Title:

SMALL PEPTIDES FOR THE TREATMENT OF ALZHEIMER'S DISEASE AND OTHER BETA-AMYLOID PROTEIN FIBRILLOGENESIS DISORDERS

This application is a continuation-in-part of US Patent Application 09/962,955 filed September 24, 2001 which is a continuation-in-part of US Patent Application 09/938,275 filed August 22, 2001, which is a continuation of US Patent Application 08/947,057 filed October 8, 1997; This application also claims priority to US Provisional Application 60/461,655 filed April 8, 2003. This invention was made with government support under 1 R43 AG17787 awarded by the National Institute on Aging. The Government has certain rights in the invention.

TECHNICAL FIELD

This invention relates to the use of small (7 to 12 mer) laminin peptides and laminin derivatives for the treatment of Alzheimer's disease and other beta-amyloid protein fibrillogenesis disorders.

BACKGROUND OF THE INVENTION

Additional background for therapeutic use of laminin, and peptide fragments of laminin, in the treatment of Alzheimer's disease and other amyloidoses can be found in US Patent Application 09/938,275 filed August 22, 2001, and in US Patent Application 09/962,955 filed September 24, 2001, the text and drawings of each of which are hereby incorporated by reference into the present application as if fully set forth herein.

Beta-Amyloid Protein as a Therapeutic Target for Alzheimer's disease

Alzheimer's disease (AD) is characterized by the deposition and accumulation of a 39-43 amino acid peptide termed the beta-amyloid protein, Aβ or β/A4 (Glenner and Wong, Biochem. Biophys. Res. Comm. 120:885-890. 1984; Masters et al, Proc. Nat. Acad. Sci. U.S.A. 82:4245-4249, 1985; Husby et al, Bull. WHO 71:105-108,1993). Aβ is derived

from larger precursor proteins termed beta amyloid precursor proteins (or APPs) of which there are several alternatively spliced variants. The most abundant forms of the APPs include proteins consisting of 695, 751 and 770 amino acids (Kitaguchi et al, Nature 331:530-532, 1988; Ponte et al, Nature 331:525-527, 1988; Tanzi et al, Nature 331:528-530, 1988). The small Aβ peptide is a major component that makes up the core of amyloid deposits called "plaques" in the brains of patients with AD. In addition, AD is characterized by the presence of numerous neurofibrillary "tangles", consisting of paired helical filaments which abnormally accumulate in the neuronal cytoplasm (Grundke-Iqbal et al Proc. Natl. Acad. Sci. U.S.A. 83:4913-4917., 1986; Kosik et al, Proc. Natl. Acad. Sci. U.S.A. 83:4044-4048, 1986; Lee et al, Science 251:675-678, 1991). The other major type of lesion found in AD brain is the accumulation of amyloid in the walls of blood vessels, both within the brain parenchyma and meningeal vessels that lie outside the brain. The amyloid deposits localized to the walls of blood vessels are referred to as cerebrovascular amyloid or congophilic angiopathy (Mandybur, J. Neuropath. Exp. Neurol. 45:79-90, 1986; Pardridge et al, J. Neurochem. 49:1394-1401, 1987). The pathological hallmarks of AD therefore are the presence of "plaques", "tangles", and cerebrovascular amyloid deposits.

For many years there has been an ongoing scientific debate as to the importance of "amyloid" in AD and whether the "plaques" and "tangles" characteristic of this disease, were a cause or merely the consequences of the disease. Recent studies indicate that amyloid is indeed a causative factor for AD and should not be regarded merely as a consequence. The Alzheimer's Aβ protein in cell culture has been shown to cause degeneration of nerve cells within a short time period (Pike et al, Br. Res. 563:311-314, 1991; J. Neurochem. 64:253-265, 1995). Studies suggest that it is the fibrillar structure, characteristic of all amyloids, that is mainly responsible for the neurologic effects. Aβ has also been found to be neurologic in slice cultures of hippocampus (Hadrian et al, Neurobiol. Aging 16:779-789, 1995) and induces nerve cell death in transgenic mice (Games et al, Nature 373:523-527, 1995; Hsiao et al, Science 274:99-102, 1996). Injection of Aβ into rat brain also causes memory impairment and neuronal dysfunction (Flood et al, Proc. Natl. Acad. Sci. U.S.A. 88:3363-3366, 1991; Br. Res. 663:271-276, 1994). Convincing evidence that Aβ amyloid is directly involved in the pathogenesis of AD comes from genetic studies. It was discovered that the increased production of Aβ could result from mutations in the gene encoding, its precursor, APP (Van Broeckhoven et al, Science

248:1120-1122, 1990; Murrell et al, Science 254:97-99, 1991; Haass et al, Nature Med. 1:1291-1296, 1995). The identification of mutations in the APP gene which causes early onset familial AD is a strong argument that Aβ and amyloid are central to the pathogenetic process underlying this disease. Four reported disease-causing mutations have now been discovered which demonstrate the importance of Aβ in causing familial AD (reviewed in Hardy, Nature Gen. 1:233-234, 1992). Lastly, recent studies suggest that a reduction in amyloid plaque load in APP transgenic mice lead to improvements in behavioral impairment and memory loss (Chen et al, Nature 408:978-982, 2000; Janus et al, Nature 408:979-982, 2000; Morgan et al, Nature 408:982-985, 2000). This is the strongest argument to date that implicates that reduction of Aβ amyloid load in brain should be a central target for the development of new and effective treatments of AD and related disorders.

Alzheimer's Disease and the Aging Population

Alzheimer's disease is a leading cause of dementia in the elderly, affecting 5-10% of the population over the age of 65 years (Jorm, A Guide to Understanding of Alzheimer's Disease and Related Disorders, New York University Press, New York, 1987). In AD, the parts of the brain essential for cognitive processes such as memory, attention, language, and reasoning degenerate. In some inherited forms of AD, onset is in middle age, but more commonly, symptoms appear from the mid-60's onward. AD today affects 4-5 million Americans, with slightly more than half of these people receiving care in many different health care institutions. The prevalence of AD and other dementias doubles every 5 years beyond the age of 65, and recent studies indicate that nearly 50% of all people age 85 and older have symptoms of AD (NIH Progress Report on AD, National Institute on Aging, 2000). Thirty-three million people of the total population of the United States are age 65 and older, and this will climb to 51 million people by the year 2025 (NIH Progress Report on AD, National Institute on Aging, 2000). The annual economic toll of AD in the United States in terms of health care expenses and lost wages of both patients and their caregivers is estimated at $80 to $100 billion (NIH Progress Report on AD, National Institute on Aging, 2000).

Laminin and Its Presence in Alzheimer's disease

Laminin is a large glycoprotein complex of 850 kDa which normally resides on the basement membrane and is produced by a variety of cells including embryonic, epithelial

and tumor cells (Foidart et al Lab. Invest. 42:336-342, 1980; Timpl, Eur. J. Biochem. 180:487-502, 1989). Laminin interacts with various extracellular matrix components including heparan sulfate proteoglycans (Riopelle and Dow, Br. Res. 525:92-100, 1990; Battaglia et al, Eur. J. Biochem. 208:359-366, 1992), heparin (Sakashita et al, FEBS Letts. 116:243-246, 1980; Del-Rosso et al, Biochem. J. 199:699-704, 1981; Skubitz et al, J. Biol. Chem. 263:4861-4868, 1988) and type IV collagen (Terranova et al, Cell 22:719-726, 1980; Rao et al, Biochem. Biophys. Res. Comm. 128:45-52, 1985; Charonis et al, J. Cell Biol. 100:1848-1853, 1985; Laurie et al, J. Mol. Biol. 189:205-216, 1986). Laminin is composed of three distinct polypeptide chains, A1, B1 and B2 (also referred to as alpha-1, β1 and gamma-1, respectively), joined in a multidomain cruciform structure possessing three short arms and one long arm (Burgeson et al, Matrix Biol. 14:209-211, 1994). Studies involving *in vitro* self-assembly and the analysis of cell-formed basement membranes have shown that the three short arms interact to form a polymer which is a part of a basement membrane network (Yurchenco et al, J. Biol. Chem. 260:7636-7644, 1985; J. Cell Biol. 117:1119-1133, 1992; Yurchenco and Cheng, J. Biol. Chem. 268:17286-17299, 1993). In addition to its role in basement membrane formation (Kleinman et al, Biochem. 22:4969-4974, 1983), laminin also plays important roles in a number of fundamental biological processes including promotion of neurite outgrowth (Lander et al, Proc. Natl. Acad. Sci. U.S.A. 82:2183-2187, 1985; Bronner-Fraser and Lallier, J. Cell Biol. 106:1321-1329, 1988) and cell adhesion (Engvall et al, J. Cell Biol. 103:2457-2465, 1986). Injury to adult brain also induces laminin production by astrocytes (Liesi et al, EMBO J. 3:683-686, 1984) indicating its role in repair processes. In AD and Down's syndrome, laminin is believed to be present in the vicinity of Aβ amyloid plaques (Perlmutter and Chui, Br. Res. Bull. 24:677-686, 1990; Murtomaki et al, J. Neurosc. Res. 32:261-273, 1992; Perlmutter et al, Micro. Res. Tech. 28:204-215, 1994). Previous studies have also indicated that the various isoforms of APP of AD bind laminin (Narindrasorasak et al, Lab. Invest. 67:643-652, 1992) and other basement membrane components, including perlecan (Narindrasorasak et al, J. Biol. Chem. 266:12878-12883, 1991), fibronectin and type IV collagen (Narindrasorasak et al, J. Biol. Chem. 270:20583-20590, 1995).

DISCLOSURE OF THE INVENTION

This application is a continuation-in-part of US Patent Application 09/962,955 filed September 24, 2001, which is a continuation-in-part of US Patent Application 09/938, 275 filed August 22, 2001, the text and drawings of each of which are hereby incorporated by reference into the present application as if fully set forth herein.

Laminin-derived peptides are disclosed which demonstrate greater efficacy in inhibiting and/or disrupting amyloid fibrils than that of iAβ5, a known peptide currently under development as a beta-amyloid disease therapeutic.

Methods are disclosed herein for the treatment and diagnosis of Alzheimer's disease and other disorders that involve the accumulation and persistence of beta-amyloid protein (Aβ), sometimes referred to herein as "beta-amyloid protein fibrillogenesis disorders". Methods are disclosed for treating Alzheimer's disease and other Aβ disorders, comprising administering to a subject or patient a therapeutically effective dose of at least one laminin globular domain-derived L- or D-form peptide, such as small 7-12 mer peptides disclosed herein, or an analog or a derivative thereof. In one exemplary embodiment, the laminin peptide which is a potent Aβ amyloid inhibitory agent is selected from the group consisting of AG73 (SEQ ID NO:1), C-16 (SEQ ID NO:2), A-13 (SEQ ID NO:3), HA3G47 (SEQ ID NO:4), HA3G58 (SEQ ID NO:5), HA3G67 (SEQ ID NO:6), HA3G74 (SEQ ID NO:7), HA3G76 (SEQ ID NO:8), HA3G79 (SEQ ID NO:9), HA3G83 (SEQ ID NO:10), A4G82 (SEQ ID NO:11), A5G15 (SEQ ID NO:12), A5G56 (SEQ ID NO:13), A5G80 (SEQ ID NO:14), A5G81 (SEQ ID NO:15), A5G82 (SEQ ID NO: 16), A5G84 (SEQ ID NO:17), A5G101 (SEQ ID NO:18), A5G109 (SEQ ID NO:19), hereinafter referred to for easy reference as Sequence Group A, but more preferably selected from the group consisting of AG73 (SEQ ID NO:1), A-13 (SEQ ID NO:3), HA3G76 (SEQ ID NO:8), A4G82 (SEQ ID NO:11), A5G81 (SEQ ID NO: 15) and A5G101 (SEQ ID NO:18), hereinafter referred to for easy reference as Sequence Group B.

The laminin peptides of the present invention may be prepared by known chemical synthetic methods or by biotechnological methods. Assays useful for the screening and identification of laminin peptide analogs as inhibitors of Aβ fibrillogenesis are also disclosed. In addition, methods are disclosed for the labeling of polypeptides derived from the invention for diagnosis of Alzheimer's and other Aβ amyloidoses.

The present invention relates to the novel and surprising discovery that laminin globular-domain derived peptides are inhibitors of Alzheimer's disease amyloidosis, and therefore have potential use for the therapeutic intervention of Alzheimer's disease and related Aβ disorders.

It is therefore an object of the present invention is to provide a method for treating Alzheimer's disease and other disorders involving the formation and persistence of Aβ, comprising the administration of laminin-derived peptides.

Another object of the present invention is to disclose specific laminin globular domain-derived peptides and other novel analogs and derivatives thereof, the administration of which comprises a method for treating Alzheimer's disease and other Aβ amyloidoses.

The invention also relates to pharmaceutical compositions comprising the laminin globular domain-derived peptides and other analogs and derivatives of such peptides, or pharmaceutically acceptable salts thereof for use in the treatment of Alzheimer's disease and other Aβ amyloidoses.

As used herein the term "laminin globular domain-derived peptide" is used to include each laminin globular domain-derived peptide which was surprisingly found to inhibit Aβ fibrillogenesis as disclosed herein, analogs, derivative and fragments thereof that retain the activity of the complex peptide. The term analogs are intended to include variants on the peptide molecule brought about, for example, homologous substitution of individual or several amino acid residues. The term derivative is used to include minor chemical changes that may be made to each of the laminin globular domain-derived peptides themselves or analogs thereof that maintain the biological activity of each of the parent peptides disclosed.

The invention also discloses methods to utilize the laminin-derived peptides as diagnostic or imaging agents for Alzheimer's disease and other Aβ amyloidoses.

The invention also discloses methods to utilize antibodies made against laminin-derived peptides as therapeutic agents for the treatment of Alzheimer's disease and other Aβ amyloid disorders.

A primary object of the present invention is to establish new therapeutic methods for Alzheimer's disease and other disease involving the accumulation of Aβ. These Aβ diseases include, but are not limited to, the amyloid associated with Alzheimer's disease

and Down's syndrome, and various forms of cerebral amyloidosis, known to those knowledgeable in the art.

A primary object of the present invention is to use laminin globular domain derived peptides as potent inhibitors of Aβ amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other Aβ amyloidoses. Laminin globular domain derived peptides include, but are not limited to, the peptides of Sequence Group A, and more preferably the peptides of Sequence Group B and/or A5G109 (SEQ ID NO:19).

Yet another object of the present invention is to use analogs or derivatives thereof of each of the laminin globular domain derived peptides as potent inhibitors of Aβ amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other Aβ amyloidoses. Laminin globular domain derived peptides include but are not limited to, the peptides of Sequence Group A, and more preferably the peptides of Sequence Group B.

Yet another object of the present invention is to use peptidomimetic compounds modeled from the laminin globular domain peptides disclosed herein, including but not limited to, the peptides of Sequence Group A.

Yet another aspect of the present invention is to make use of laminin globular domain-derived peptides including, but not limited to, the peptides of Sequence Group A, and fragments or analogs thereof, as potential therapeutics to inhibit the deposition, formation and accumulation of fibrillar amyloid in Alzheimer's disease and other Aβ amyloidosis disorders, and to enhance the clearance and/or removal of pre-formed amyloid deposits in brain (for Alzheimer's disease and Down's syndrome and other Aβ amyloidoses).

Yet another object of the present invention is to use the laminin globular domain-derived peptides of the present invention, and all constituents, analogs or variants thereof, including peptides which have at least 70% identity to the sequences disclosed herein. Specific laminin globular domain-derived peptides as described above may be derived from any species including, but are not limited to, human, murine, bovine, porcine, and/or equine species.

Yet another object of the present invention is to use laminin globular domain-derived peptides as described herein as a specific indicator for the presence and extent of

laminin breakdown in brain by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum and stool.

Yet another object of the present invention is to make use of peptides or analogs or derivatives thereof as described herein, including but not limited to, the peptides of Sequence Group A, as potential blocking therapeutics for the interaction of laminin and laminin-derived fragments in a number of biological processes and diseases (such as in Alzheimer's disease, Down's syndrome and other amyloid diseases).

Another object of the present invention is to use pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, and sterile packaged powders, which contain laminin globular domain-derived peptides, including but not limited to, the peptides of Sequence Group A, and analogs, derivatives or fragments thereof, to treat patients with Alzheimer's disease and other Aβ amyloidoses.

Yet another object of the present invention is to provide compositions and methods involving administering to a subject a therapeutic dose of laminin globular domain-derived peptides, which inhibit Aβ amyloid deposition, including but not limited to, the peptides of Sequence Group A, and analogs, derivatives or fragments thereof. Accordingly, the compositions and methods of the invention are useful for inhibiting amyloidosis in disorders in which amyloid deposition occurs. The peptides of the invention can be used therapeutically to treat amyloidosis or can be used prophylactically in a subject susceptible to amyloidosis. The methods of the invention are based, at least in part, in directly inhibiting Aβ amyloid fibril formation, and/or causing dissolution of pre-formed Aβ amyloid fibrils.

Yet another object of the present invention is to provide pharmaceutical compositions for treating Aβ amyloidosis. The pharmaceutical compositions include a therapeutic compound of the invention in an amount effective to inhibit Aβ amyloid deposition and a pharmaceutically acceptable vehicle.

Yet a further aspect of the present invention is to use anti-idiotypic antibodies to laminin-derived protein fragments and/or laminin-derived polypeptides as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other Aβ amyloidoses.

Another aspect of the invention is to provide new and novel polyclonal and/or monoclonal peptide antibodies which can be utilized in a number of *in vitro* assays to specifically detect Aβ-binding laminin derived protein fragments and/or Aβ-binding laminin derived polypeptides in human tissues and/or biological fluids. Polyclonal or monoclonal antibodies that are made specifically against a peptide portion or fragment of laminin which interacts with Aβ can be utilized to detect and quantify amyloid disease specific laminin fragments in human tissues and/or biological fluids. These antibodies can be made by administering the peptides in antigenic form to a suitable host. Polyclonal or monoclonal antibodies may be prepared by standard techniques known to those skilled in the art.

Another object of the present invention is to use laminin-derived polypeptides referred to above, for the detection and specific localization of laminin peptides important in the amyloid diseases in human tissues, cells, and/or cell culture using standard immunohistochemical techniques.

Yet another aspect of the present invention is to use antibodies recognizing any of the Aβ-binding laminin fragments, and/or laminin-derived polypeptides including, but not limited to, the peptides of Sequence Group A, and analogs, derivatives or fragments thereof, for *in vivo* labeling; for example, with a radionucleotide, for radioimaging to be utilized for *in vivo* diagnosis, and/or for *in vitro* diagnosis.

Another object of the present invention is to use Aβ-binding laminin-derived polypeptides or fragments thereof, in conjunction with polyclonal and/or monoclonal antibodies generated against these peptide fragments, using *in vitro* assays to detect amyloid disease specific autoantibodies in human biological fluids. Specific assay systems can be utilized to not only detect the presence of autoantibodies against Aβ-binding laminin-derived protein fragments or polypeptides thereof in biological fluids, but also to monitor the progression of disease by following elevation or diminution of laminin protein fragments and/or laminin-derived polypeptide autoantibody levels.

Another aspect of the invention is to utilize laminin-derived protein fragments and/or laminin-derived polypeptide antibodies and/or molecular biology probes for the detection of these laminin derivatives in human tissues in the amyloid diseases.

Yet another object of the present invention is to use the laminin-derived protein fragments or polypeptides of the present invention in each of the various therapeutic and

diagnostic applications described above. The laminin-derived protein fragments include, but are not limited to, a ~55 kDa fragment of laminin generated by trypsin digestion, a ~55 kDa fragment of laminin generated by elastase digestion, and a ~30 kDa fragment of laminin generated by trypsin digestion. The laminin-derived polypeptides include, but are not limited to the peptides of Sequence Group A, and analogs, derivatives or fragments thereof, including peptides which have at least 70% identity to the sequences disclosed herein. Specific laminin-derived protein fragments or peptides as described above may be derived from any species including, but are not limited to, human, murine, bovine, porcine, and/or equine species.

Another object of the invention is to provide polyclonal and/or monoclonal peptide antibodies that can be utilized in a number of *in vitro* assays to specifically detect laminin protein fragments or polypeptides in human tissues and/or biological fluids. Polyclonal or monoclonal antibodies made specifically against a peptide portion or fragment of any of the laminin fragments or polypeptides described herein can be utilized to detect and quantify laminin-derived protein fragments or laminin-derived polypeptides in human tissues and/or biological fluids. These antibodies can be made by isolating and administering the laminin-derived fragments and/or polypeptides in antigenic form to a suitable host. Polyclonal or monoclonal antibodies may be prepared by standard techniques by one skilled in the art.

Yet another object of the present invention is to use laminin-derived fragment or polypeptide-derived antibodies as described herein as a specific indicator for the presence and extent of laminin breakdown in brain by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to use laminin-derived fragment or polypeptide antibodies as described herein as a specific indicator for the presence, extent and/or progression of Alzheimer's disease and/or other brain amyloidoses by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to use laminin-derived fragment or polypeptide-derived antibodies as described herein as a specific indicator for the presence and extent of laminin breakdown in systemic organs by monitoring biological fluids

including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to use laminin-derived fragment or polypeptide antibodies as described herein as a specific indicator for the presence and extent of amyloidosis in type II diabetes by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to use laminin-derived fragment or polypeptide antibodies as described herein as a specific indicator for the presence and extent of amyloidosis in other systemic amyloidoses by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to make use of peptides or fragments of laminin as described herein, including but not limited to, the peptides of Sequence Group A, and fragments thereof, as potential blocking therapeutics for the interaction of laminin and laminin-derived fragments in a number of biological processes and diseases (such as in Alzheimer's disease and other amyloid diseases described herein).

Yet another object of the invention is to utilize specific laminin-derived fragment or polypeptide antibodies, as described herein, for the detection of these laminin fragments in human tissues in the amyloid diseases.

Preferred pharmaceutical compositions have at least one laminin peptide or fragment thereof selected from the Sequence Group A consisting of AG73 (SEQ ID NO:1), C-16 (SEQ ID NO:2), A-13 (SEQ ID NO:3), HA3G47 (SEQ ID NO:4), HA3G58 (SEQ ID NO:5), HA3G67 (SEQ ID NO:6), HA3G74 (SEQ ID NO:7), HA3G76 (SEQ ID NO:8), HA3G79 (SEQ ID NO:9), HA3G83 (SEQ ID NO:10), A4G82 (SEQ ID NO:11), A5G15 (SEQ ID NO:12), A5G56 (SEQ ID NO:13), A5G80 (SEQ ID NO:14), A5G81 (SEQ ID NO:15), A5G82 (SEQ ID NO: 16), A5G84 (SEQ ID NO:17), A5G101 (SEQ ID NO:18) and A5G109 (SEQ ID NO:19).

In preferred embodiments the compositions (all from Sequence Group B) have the structure Arg-Lys-Arg-Leu-Gln-Val-Gln-Leu- Ser-Ile-Arg-Thr (SEQ ID NO: 1) or Arg-Gln-Val-Phe-Gln-Val-Ala-Tyr Ile-Ile-Ile-Lys-Ala (SEQ ID NO:3) or Tyr-Leu-Ser-Lys-Gly-Arg-Leu-Val- Phe-Ala-Leu-Gly (SEQ ID NO:8) or Thr-Leu-Phe-Leu-Ala-His-Gly-Arg- Leu-Val-

Phe-Met (SEQ ID NO:11) or Ala-Gly-Gln-Trp-His-Arg-Val-Ser- Val-Arg-Trp-Gly (SEQ ID NO:15) or Asp-Gly-Arg-Trp-His-Arg-Val-Ala- Val-Ile-Met-Gly (SEQ ID NO:18).

Alternate preferred compositions and pharmaceutical compositions have at least one laminin peptide or fragment thereof selected from the Sequence Group D consisting of DP2 D-A13, DP3 D-HA3G76, DP4 D-A4G82, DP5 D-A5G81, DP6 D-A5G101, DP7 D-HA3G47, DP8 D-HA3G58, DP9 D-HA3G74, DP10 D-HA3G83, DP11 D-A5G82, DP13 D-R-AG73, DP14 D-R-A13, DP15 D-R-HA3G76, DP16 D-R-A4G82, and DP17 D-R-A5G81, and more preferably from the Sequence Group E consisting of DP2 D-A13, DP4 D-A4G82, DP5 D-A5G81, DP6 D-A5G101, DP8 D-HA3G58, DP10 D-HA3G83, DP14 D-R-A13, DP15 D-R-HA3G76 and DP16 D-R-A4G82.

In any of the above structures or sequences, the individual amino acids may be either L- or D-amino acids. The pharmaceutical composition have a therapeutically effective amount of any of the above structures or sequences, preferably together with a pharmaceutically acceptable carrier, diluent or excipient.

In any of the above structures or sequences, the nomenclature or symbolic representation of any or all of the individual amino acids may be given by either the standard 3-letter abbreviation for the amino acid, or the standard single letter code for the amino acid, and sometimes both in appropriate cases.

Preferred pharmaceutical agents for treating Aβ amyloidosis in a patient have a therapeutically effective amount of a polypeptide selected from Sequence Group B or A5G109 (SEQ ID NO:19), and have an Aβ amyloid inhibitory activity or efficacy greater than 30%, as compared to duly established controls, such as patients who do not received the preferred pharmaceutical agent.

An important Aβ amyloidosis to which the disclosed therapeutics are addressed is Alzheimer's disease. A preferred therapeutically effect amount of disclosed polypeptide is a dosage in the range of from about 10 μg to about 50 mg/kg body weight/per day, and more preferably in the range of from about 100μg to about 10 mg/kg body weight per day.

The pharmaceutical agent may advantageously be administered in a parenterally injectable or infusible form or orally.

A method is also disclosed to diagnose a disease or susceptibility to Aβ amyloidosis related to the level of laminin-derived polypeptides. First the levels of laminin-derived polypeptides in a sample are determined, whereby the levels are indicative of the presence

of Aβ amyloidosis, susceptibility to Aβ amyloidosis, or progression of Aβ amyloidosis. In preferred methods the laminin-derived polypeptides are selected from the group consisting of Sequence Group B and/or A5G109 (SEQ ID NO:19).

The sample assayed may be a biological fluid, and the biological fluid may be serum derived from humans.

A method of making an antibody is also disclosed, the method producing antibodies from a peptide sequence selected from the group consisting of Sequence Group B and/or A5G109 (SEQ ID NO:19), and fragments thereof. The method preferably includes production of at least one type of antibody selected from the group consisting of polyclonal, monoclonal, chimeric, and anti-idiotypic antibodies and monitoring a biological fluid for the presence and extent of laminin-derived polypeptides as an indicator for the extent of an amyloid disease and radiolabeling the antibodies for radioimaging or in vivo diagnosis for detection of laminin-derived protein fragments or laminin-derived polypeptides.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention.

FIGURE 1 is a graph demonstrating an inhibitory effect of Aβ amyloid deposition into rodent hippocampus by laminin.

FIGURE 2 is a copy of a black and white photograph of a Coomassie blue stained gel demonstrating purification and isolation of fragments of laminin which strongly interact with Aβ.

FIGURE 3 is a graph demonstrating the strong binding interaction of Alzheimer's Aβ to the ~55 kilodalton laminin fragment. A single dissociation constant with a $K_d = 2.0 \times 10^{-9}$ was determined.

FIGURE 4 is a graph demonstrating the inhibition of Alzheimer's Aβ fibril formation by selected fragments disclosed herein.

FIGURE 5 is a schematic representation of the sequence of human alpha-3 chain globular domain peptides disclosed herein.

FIGURE 6 is a schematic representation of the sequence of murine alpha-4 chain globular domain peptides disclosed herein.

FIGURE 7 is a schematic representation of the sequence of murine alpha-5 chain globular domain peptides disclosed herein.

FIGURE 8 is a table which includes laminin globular domain-derived peptides which can disrupt/ disassemble pre-formed Alzheimer's Aβ 1-40 fibrils.

FIGURE 9 is a graph demonstrating further testing of selected laminin globular-domain derived peptides against pre-formed Alzheimer's Aβ 1-42 fibrils.

FIGURE 10 is a graph demonstrating dose-dependent disruption/disassembly of pre-formed Aβ 1-42 fibrils by laminin globular domain-derived peptides.

[Designation Figure 11 intentionally left unused at this time]

FIGURES 12a-f are laminin-derived peptide sequences for 12-13 mer peptides DP1-18 and LP19-25, and 7 mer peptides DP 26-49.

FIGURE 13 is a graph of *in vitro* screening of peptides DP1-6, DP13-18, and LP19, 25 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils (Alzheimer's Disease) as assessed by a Thioflavin T fluorometry assay.

FIGURE 14 is a graph of *in vitro* screening of peptides DP1-6, DP13-18, and LP19, 25 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by a Congo Red binding assay.

FIGURE 15 is a graph of *in vitro* screening of peptides DP2, 14, 7-12, LP 19, 25, AG73, A4G82, iAβ5, and A5G101 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by a Thioflavin T fluorometry assay.

FIGURE 16 is a graph of *in vitro* screening of peptides DP2, 14, 7-12, LP 19, 25, AG73, A4G82, iAβ5, and A5G101 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by a Congo Red binding assay.

FIGURE 17a-d are graphs of peptides DP1-2 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by circular dichroism spectroscopy (CD).

FIGURE 18a-d are graphs of peptides DP3-4 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

FIGURE 19a-d are graphs of peptides DP5-6 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

FIGURE 20a-d are graphs of peptides DP7-8 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

FIGURE 21a-d are graphs of peptides DP9-10 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

FIGURE 22a-d are graphs of peptides DP11-12 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD

FIGURE 23a-d are graphs of peptides DP13-14 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

FIGURE 24a-d are graphs of peptides DP15-16 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

FIGURE 25a-d are graphs of peptides DP17-18 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

FIGURE 26a-b are graphs of peptide LP19 demonstrating its effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

BEST MODE OF CARRYING OUT THE INVENTION

Accordingly the use of laminin-derived peptides for the treatment of Alzheimer's disease and other Aβ amyloidoses is disclosed. Specifically observed and isolated laminin globular domain-derived peptides disclosed herein have the ability to inhibit Aβ fibril formation, and cause a disruption of pre-formed Aβ amyloid fibrils, and therefore possess therapeutic potential in the treatment of Alzheimer's disease and other disorders involving the formation, deposition, accumulation and persistence of Aβ.

Pharmaceutically acceptable salts of the peptides disclosed in the present invention include both salts of the carboxy groups and the acid addition salts of the amino groups of the peptide molecule. Salts of the carboxy groups may be formed by methods known in the art and include inorganic salts such as sodium, calcium ammonium, ferric or zinc salts and the like and salts with organic bases such as those formed with amines such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include salts with mineral acids such as hydrochloric acid and sulphuric acid and salts of organic acids such as acetic acid or oxalic acid.

The pharmaceutical composition may contain laminin-derived peptides such as those disclosed herein as unique peptides or in polymerized or conjugated form attached to macromolecular carriers or polymers. The compositions may optionally contain

pharmaceutically acceptable excipients. In an alternative embodiment the composition may contain the laminin-derived peptide alone.

The route of administration includes oral, intravenous, intra-peritoneal, intra-muscular, subcutaneous, intra-articular, intra-nasal, intra-thecal, intra-dermal, transdermal or by inhalation. An effective dose of each of the laminin-derived peptides disclosed herein as potential therapeutics for use in treating Aβ amyloidosis in Alzheimer's disease and other disorders be from about 1μg to 500 mg/kg body weight, per single administration, which may readily be determined by one skilled in the art. The dosage depends upon the age, sex, health, and weight of the recipient, kind of concurrent therapy, if any, and frequency of treatment.

As used herein the laminin-derived polypeptides of the present invention may consist of -L amino acid, -D amino acids or a mixture of both forms. Amino acids in nature usually consist of -L amino acids. However, substitution with -D amino acids may demonstrate enhanced Aβ amyloid inhibitory activity, enhanced bioavailability due to less degradation in biological fluids (such as plasma), and enhanced penetration across the blood-brain-barrier. Polypeptides having an identical amino acid sequence to that found within a parent peptide but which all or part of the L-amino acids have been substituted with D-amino acids is part of the present invention for the development of therapeutics to treat Alzheimer's disease and other Aβ amyloidoses.

The -L or -D amino acids of the laminin-derived polypeptides of the present invention are further intended to include other peptide modifications, including derivatives, analogs and mimetics, that retain the ability of the polypeptides to inhibit Aβ amyloidosis as described herein. The terms "analog", "derivative" and "mimetic" as used herein are intended to include molecules which mimic the chemical structure of a L or D-peptidic structure, and retain the functional properties of a L- or D-peptidic structure. Approaches to designing peptide analogs, derivatives and mimetics are known in the art. For example, see P.S. Farmer, in Drug Design, E.J. Ariens, ed., Academic Press, New York, 1980, v. 10, pp. 119-143; Ball and Alewood, J. Mol. Recognition 3:55, 1990; Morgan and Gainor, Ann. Rep. Med. Chem. 24:243, 1989; and Freidinger, Trends Pharmacol. Sci. 10:270, 1989. See also Sawyer, "Peptidomimetic design and chemical approaches to peptide metabolism", in MD Taylor and GL Amidon, eds., in Peptide-Based Drug Design: Controlling Transport and Metabolism, Ch. 17, 1995; Smith et al, J. Am. Chem. Soc.

117:11113-11123, 1995; Smith et al, J. Am. Chem. Soc. 116:9947-9962, 1994; and Hirschman et al, J. Am. Chem. Soc. 115:12550-12568, 1993.

As used herein, a "derivative" of a therapeutic compound (e.g. a peptide or polypeptide) refers to a form of the peptide in which one or more reaction groups of the compound have been derivatized with a substituent group. Examples of peptide derivatives include peptides in which an amino acid side chain, the peptide backbone, or the amino- or carboxy-terminus has been derivatized (e.g., peptidic compounds with methylated amide linkages). As used herein an analog of a therapeutic compound refers to a compound which retains chemical structures necessary for functional activity (i.e. Aβ inhibitory activity), yet which also contains certain chemical structures which differ from the parent peptide. An example of an analog of a naturally occurring peptide is a peptide which includes one or more non-naturally -occurring amino acids. As used herein, a "mimetic" of a compound refers to a compound in which chemical structures of the compound are necessary for functional activity have been replaced with other chemical structures which mimic the conformation of the compound or peptides thereof. Examples of peptidomimetics include peptide compounds in which the peptide backbone is substituted with one or more benzodiazepine molecules (see James et al, Science 260:1937-1942, 1993).

Analogs of the polypeptide compounds of the invention are intended to include compounds in which one or more L- or -D amino acids of the peptide structure are substituted with a homologous amino acid such that the properties of the original polypeptide are maintained. Preferably conservative amino acid substitutions are made at one or more amino acid residues. A "conservative amino acid substitution" in one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagines, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), β-branched side chains (e.g., threonine, valine, isoleucine), and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Non-limiting examples of homologous substitutions that can be made in the peptidic structures of the invention include substitution of

phenylalanine with tyrosine, leucine with valine, or other natural or non-natural amino acid having an aliphatic side chain and/or substitution of valine with leucine or other natural or non-natural amino acid having an aliphatic side chain.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Preferably, the carrier is suitable for administration into the central nervous system (e.g. intraspinally or intracerebrally). Alternatively, the carrier can be suitable for intravenous, intraperitoneal or intramuscular administration. In another embodiment, the carrier is suitable for oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is compatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

As used here in "Aβ amyloidoses" refers to amyloid diseases which involve the formation, deposition, accumulation and/or persistence of Aβ (i.e. beta-amyloid protein), including but not limited to Aβ containing 39-43 amino acids in length, but more preferably, Aβ 1-40 (SEQ ID NO:36), or Aβ 1-42 (SEQ ID NO:37), and mixtures or fragments thereof.

"Aβ amyloidoses" and "Aβ fibrillogenesis diseases" include, but are not limited to Alzheimer's disease, Down's syndrome, forms of familial amyloidosis, cerebrovascular amyloidosis and cerebral hemorrhage, cystatin C amyloid angiopathy, hereditary cerebral hemorrhage with amyloidosis (Dutch type), hereditary cerebral hemorrhage with amyloidosis (Icelandic type), and inclusion body myositis.

These and other features and advantages of the present invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying figures which are illustrative of embodiments of the invention only, and are not meant to limit the scope of the invention.

Figure 1 is a graph demonstrating inhibition of fibrillar Aβ amyloid deposition into rodent hippocampus by laminin. Laminin caused a significant 90% inhibition of Aβ amyloid deposition in brain.

Figure 2 is a black and white photograph of a Coomassie blue stained gel demonstrating purification and isolation of a ~55 kilodalton fragment of laminin, and a ~30 kilodalton subfragment of laminin, identified as fragments of laminin which strongly interact with Aβ.

Figure 3 is a graph demonstrating the strong binding interaction of Alzheimer's Aβ to the ~55 kilodalton laminin fragment. A single dissociation constant with a K_d = 2.0 X 10^{-9} was determined.

Figure 4 is a graph demonstrating the inhibition of Alzheimer's Aβ fibril formation by various protease-generated laminin fragments. Intact laminin, ~55 kilodalton and ~30 kilodalton laminin-fragments obtained by trypsin digestion, and a ~55 kilodalton fragment of laminin obtained by elastase digestion, all significantly inhibited Alzheimer's Aβ fibril formation at 3 and 7 days.

Figure 5 is a schematic that demonstrates the sequence of peptides derived from the human alpha-3 chain globular domain that was used for screening for Alzheimer's Aβ amyloid inhibitory activity. A total of 49 12-14 amino acid peptides (labeled A3G40 to A3G88) were synthesized and used for screening studies.

Figure 6 is a schematic that demonstrates the sequence of peptides derived from the mouse alpha-4 chain globular domain that was used for screening for Alzheimer's Aβ amyloid inhibitory activity. A total of 117 12-14 amino acid peptides (labeled A4G-1 to A4G-116) were synthesized and used for screening studies.

Figure 7 is a schematic that demonstrates the sequence of peptides derived from the mouse alpha-5 chain globular domain that was used for screening for Alzheimer's Aβ amyloid inhibitory activity. A total of 133 12-14 amino acid peptides (labeled A5G-1 to A5G-113) were synthesized and used for screening studies.

Figure 8 is a table that identifies the laminin globular domain-derived peptides which can disrupt/ disassemble pre-formed Alzheimer's Aβ 1-40 fibrils.

Figure 9 is a graph demonstrating the further testing of selected laminin globular-domain derived peptides against pre-formed Alzheimer's Aβ 1-42 fibrils.

Figure 10 is a graph demonstrating dose-dependent disruption/disassembly of pre-formed Aβ 1-42 fibrils by laminin globular domain-derived peptides.

Figures 12a-f are laminin peptide sequences for 12-13 mer peptides DP1-18 and LP19-25, and 7 mer peptides DP 26-49.

Figure 13 is a graph of *in vitro* screening of peptides DP1-6, DP13-18, and LP19, 25 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils (Alzheimer's Disease) as assessed by a Thioflavin T fluorometry assay.

Figure 14 is a graph of *in vitro* screening of peptides DP1-6, DP13-18, and LP19, 25 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by a Congo Red binding assay.

Figure 15 is a graph of *in vitro* screening of peptides DP2, 14, 7-12, LP 19, 25, AG73, A4G82, iAβ5, and A5G101 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by a Thioflavin T fluorometry assay.

Figure 16 is a graph of *in vitro* screening of peptides DP2, 14, 7-12, LP 19, 25, AG73, A4G82, iAβ5, and A5G101 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by a Congo Red binding assay.

Figure 17a-d are graphs of peptides DP1-2 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by circular dichroism spectroscopy (CD).

Figure 18a-d are graphs of peptides DP3-4 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

Figure 19a-d are graphs of peptides DP5-6 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

Figure 20a-d are graphs of peptides DP7-8 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

Figure 21a-d are graphs of peptides DP9-10 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

Figure 22a-d are graphs of peptides DP11-12 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD

Figure 23a-d are graphs of peptides DP13-14 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

Figure 24a-d are graphs of peptides DP15-16 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

Figure 25a-d are graphs of peptides DP17-18 demonstrating their effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

Figure 26a-b are graphs of peptide LP19 demonstrating its effects on inhibition of Ab 1-42 amyloid fibrils as assessed by CD.

Examples

The following examples are provided to disclose in detail preferred embodiments of the potent inhibitory effects of laminin fragments, and laminin globular domain-derived peptides on Aβ fibrillogenesis. However, it should not be construed that the invention is limited to these specific examples.

Example 1

Screening of Peptides from the Globular Domain Regions of Different Laminin Chains

A series of overlapping 12-14 amino acid peptides against the globular domain regions of the alpha-1, alpha-3 (Figure 5), alpha-4 (Figure 6) and alpha-5 (Figure 7) chain of laminin were synthesized. More than 300 (12-14 amino acid) peptides corresponding to the globular domain regions of the various laminin chains were synthesized manually using the 9-fluorenylmethoxy-carbonyl (FMOC) method and C-terminal amides. The respective amino acids were condensed manually in a stepwise manner using 4-(2",4"-dimethoxyphenyl-FMOC-amino-methyl)-phenoxy resin (Rink, Tetrahedron Lett. 28:3787-3790, 1987). The amino acid side chain protecting groups were removed as described previously (Nomizu et al, J. Biol. Chem. 269:30386-30392,1994; J. Biol. Chem. 270: 20583-20590, 1995). The resulting protected synthetic peptide resins were de-protected and cleaved from the resins using trifluoroacetic acid-thianisole-m-cresol ethanedithiol-H_2O (80:5:5:5:5) at 20°C for 3 hours. Crude peptides were then precipitated and washed with ethyl ether and purified by reverse phase HPLC using a Vydac 5C18 column with a gradient of water/acetonitrile containing 0.1 % trifluoroacetic acid. The purity of the peptides was confirmed by analytical HPLC. The identity of each peptide was confirmed using a Sciex API IIIE triple quadruple ion spray mass spectrometer (Otaka et al J. Org. Chem. 60:3967-3974, 1995). More than 300 peptides were synthesized for Aβ amyloid inhibitory activity screening using Thioflavin T fluorometry (Castillo et al J. Neurochem. 69:2452-2465, 2000).For initial screening studies, 25 μM of pre-formed Aβ 1-40 fibrils were incubated for 7 days with various 12-14 amino acid laminin globular domain-derived

peptides at an Aβ:peptide molar ratio of 1:6. Of 300 peptides screened, only 30 peptides (listed in Figure 8) were found to demonstrate a disruption/disassembly greater than 20%. The significance was determined using the paired t-test and comparing fluorescence units ± S.D. (n=3) of Aβ alone versus Aβ + laminin-derived peptides.

Example 2

Laminin Globular Domain Peptides that Disrupt/Disassemble Pre-formed Aβ Fibrils

Figure 8 lists 30 laminin globular domain peptides that were able to cause a disruption/disassembly of pre-formed Aβ 1-42 fibrils. These Alzheimer's amyloid inhibitor peptides included 3 peptides from the laminin alpha-1 chain globular domain (peptides AG73, LAM-L, and A13; Figure 8), 1 peptide from the laminin gamma-1 chain (peptide C-16; Figure 8), 11 peptides from the laminin alpha-3 chain globular domain (peptides A3; HA3G45; HA3G47; HA3G58; HA3G67; HAG371; HAG374; HAG375; HAG376; HAG379, and HAG383; Figure 8), 2 peptides from the laminin alpha-4 chain globular domain (peptides A4G31 and A4G82; Figure 8), and 12 peptides from the laminin alpha-5 chain globular domain (peptides A5; A5G15; A5G35; A5G46; A5G46; A5G56; A5G71; A5G80; A5G81; A5G82; A5G84; A5G101; A5G109 and A5G110; Figure 8).

Example 3

Further Testing of Selected Laminin Globular Domain-Derived Peptides for Aβ 1-42 Amyloid Fibril Inhibitory Activity

From the screening results shown in Example 2 and Figure 8, 19 peptides (out of >300 screened) were identified that were effective in causing a >25% disruption/disassembly of pre-formed Aβ 1-40 fibrils. These included peptides AG73 (SEQ ID NO:1), C-16 (SEQ ID NO: 2), A-13 (SEQ ID NO:3), HA3G47 (SEQ ID NO: 4), HA3G58 (SEQ ID NO: 5), HA3G67 (SEQ ID NO: 6), HA3G74 (SEQ ID NO: 7), HA3G76 (SEQ ID NO: 8), HA3G79 (SEQ ID NO: 9), HA3G83 (SEQ ID NO: 10), A4G82 (SEQ ID NO: 11), A5G15 (SEQ ID NO: 12), A5G56 (SEQ ID NO: 13), A5G80 (SEQ ID NO: 14), A5G81 (SEQ ID NO: 15), A5G82 (SEQ ID NO: 16), A5G84 (SEQ ID NO:17), A5G101 (SEQ ID NO:18) and A5G109 (SEQ ID NO: 19) (Sequence Group A).

These selected laminin globular domain-derived peptides were then tested for their effectiveness to also disrupt/disassemble pre-formed Aβ 1-42 fibrils (Figure 9). In this latter study, selected laminin globular domain-derived peptides were incubated with pre-

formed Aβ 1-42 fibrils at an Aβ:peptide molar ratio of 1:10. Direct comparisons were made to iAβ5, a 5 amino-acid (LPFFD) Aβ inhibitor previously identified as a potent inhibitor of Aβ fibrillogenesis (Soto et al, Nature Med. 4:822-826, 1998). The results demonstrate that six laminin globular domain-derived peptides were significantly more effective than iAβ5 in causing a disruption/disassembly of preformed Aβ 1-42 fibrils (Figure 9). These laminin-derived peptides included peptides from the laminin alpha-1 chain [(i.e. AG73 - SEQ ID NO:1; A13 -SEQ ID NO 3), the laminin alpha-3 chain (i.e. HA3G76 - SEQ ID NO:8), the laminin alpha-4 chain (i.e. A4G82 - SEQ ID NO: 11) and the laminin alpha-5 chain (i.e.A5G81- SEQ ID NO:15; A5G101 - SEQ ID NO: 18). It should be noted that two of these Aβ inhibiting peptides were derived from the globular domain of the laminin alpha-1 chain, and the more effective of these two peptides (i.e. AG73 -SEQ ID NO:1) was precisely located within the 4th globular domain of the laminin-1 chain, and found to bind very tightly to Aβ (Fig. 8). In our studies (described above), the AG73 (SEQ ID NO:1) peptide disrupted Aβ 1-42 fibrils by 81% when used at an Aβ:peptide molar ratio of 1:10. In comparison, this peptide was 31% more effective than the previously described iAβ5 peptide (Soto et al, Nature Med. 4:822-826 1998), which in our studies only dissociated pre-formed Aβ 1-42 fibrils by 50%. At an Aβ:peptide molar ratio of 1:2, the AG73 peptide (SEQ ID NO:1) was also found to disrupt/disassemble pre-formed Aβ 1-42 fibrils by 72 %, whereas the iAβ5 peptide only caused a 27% disruption/disassembly (Figure 9). The other laminin fragment reported in the literature (Monji et al, Neurosc. Lett. 251:65-68, 1998) required an Aβ:peptide molar ratio of 1:10 to obtain a 50% inhibition of Aβ fibril formation, whereas our newly identified AG73 peptide (SEQ ID NO:1) only required an Aβ:peptide molar ratio of 1:1 to achieve the same level of inhibition. Assuming that the 12-amino acid peptide, AG73 (SEQ ID NO:1), represents a single-site of Aβ binding, we can be confident that we are close to theoretically optimum inhibition. During this screening process, we also identified 5 other peptides in the alpha 3, 4, and 5 chains that were most effective in disrupting/ causing a disassembly of pre-formed Aβ fibrils (see Figure 8).

Example 4

Dose-Dependent Disassembly of Pre-formed Aβ 1-42 Fibrils by Laminin Globular Domain-Derived Peptides

The next study was implemented to determine whether the six selected laminin globular domain-derived peptides were capable of causing a dose-dependent disassembly/disruption of pre-formed AD amyloid fibrils containing Aβ 1-42. As shown in Figure 10, disruption of pre-formed AD amyloid fibrils by all six selected laminin-derived peptides occurred following a 7-day incubation period, and in a dose-dependent manner. Significant ($p < 0.001$) disassembly/ disruption of pre-formed AD amyloid fibrils containing Aβ 1-42 was observed in the presence of laminin globular domain-derived peptides and iAβ5. Whereas iAβ5 was effective at all molar ratios tested, the selected laminin peptides were more potent ($p < 0.05$) than iAβ5 at Aβ:peptide molar ratios of 1:2 and 1:10, with the laminin globular domain derived-peptides showing a range of inhibition from 53-87 % compared to a range of inhibition from 27-50 % for iAβ5. At an Aβ:peptide molar ratio of 1:20, the laminin-derived peptides AG73 (SEQ ID NO:1), HA3G76 (SEQ ID NO:8), A5G81 (SEQ ID NO:15), A4G82 (SEQ ID NO:11) were all still significantly ($p < 0.001$) more effective than iAβ5. Both A5G101 (SEQ ID NO:18) and A13 (SEQ ID NO:3) have a similar effectiveness to iAβ5 at an Aβ:peptide molar ratio of 1:20. This study therefore demonstrated that we have identified specific candidate laminin globular domain-derived peptides that caused a disassembly/ disruption of pre-formed AD amyloid fibrils in a dose-dependent manner following a 7-day incubation.

Example 5

Synthesis of Laminin Globular Domain Analogs

Laminin globular domain-derived peptides (as described above) can be produced in both the L- and D-amino acid forms. In addition, truncated peptides and peptide analogs can be assembled for use as potential potent therapeutic peptides for the treatment of Aβ fibrillogenesis in Alzheimer's disease and related disorders. These peptides can be produced by methods well known to one skilled in the art. For example, L- and D-laminin globular domain-derived peptides could be synthesized on peptide synthesizers known to those skilled in the art, such as an Advanced ChemTech Model 396 multiple peptide synthesizer (Louisville, KY) using an automated protocol established by the manufacturer for 0.025 mmole scale synthesis. Double couplings are performed on all

cycles using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/N,N-diisopropylethylamine (DIEA)/HOBt/FMOC-AA in four-fold excess for 30 minutes followed by DIC/HOBt/FMOC-AA in fourfold excess for 45 minutes. The peptide is then deprotected and removed from the resin by treatment with TFA/water (95%/5%) for 3 hours and then precipitated with cold ether. The resulting solid is pelleted by centrifugation (2400 rpm x 10 min), and the ether is discarded. The solid is then be re-suspended in ether and re-centrifuged for the second time after which the ether is decanted for the second time. The solid is dissolved in 10 % acetic acid and lyophilized to dryness (~ 30 mg for 12 amino acid peptides; 18 mg for 7 amino acid peptides). The crude peptide is purified by preparative HPLC using instruments known to those skilled in the art such as a HP 1100 series with diode array detector, with a Vydac C18 column (21 x 250 mm) using a 15%-40 % acetonitrile gradient over 80 minutes, at a flow rate of 5 ml/min. The primary fraction is collected and re-analyzed for purity using analytical HPLC to ensure a single symmetrical peak at all wavelengths. The confirmation of structures and sequences is based on comparison of predicted molecular weights to molecular weights obtained by mass spectroscopy. These analyses are performed using instruments known to those skilled in the art, such as a Sciex API IIIE triple quadruple ion spray mass spectrometer, for example.

Laminin globular domain derived 12-13 amino acid peptides showing the more favorable Aβ amyloid inhibitory activity as described in Examples above include, but are not limited to:

1) AG73 RKRLQVQLSIRT (Arg-Lys-Arg-Leu-Gln-Val-Gln-Leu-Ser-Ile-Arg-Thr) SEQ ID NO 1

2) A13 RQVFQVAYIIIKA (Arg-Gln-Val-Phe-Gln-Val-Ala-Tyr-Ile-Ile-Ile-Lys-Ala) SEQ ID NO 3

3) HA3G76 YLSKGRLVFALG (Tyr-Leu-Ser-Lys-Gly-Arg-Leu-Val-Phe-Ala-Leu-Gly) SEQ ID NO 8

4) A4G82 TLFLAHGRLVFM(Thr-Leu-Phe-Leu-Ala-His-Gly-Arg-Leu-Val-Phe-Met) SEQ ID NO 11

5) A5G81 AGQWHRVSVRWG (Ala-Gly-Gln-Trp-His-Arg-Val-Ser-Val-Arg-Trp-Gly) SEQ ID NO 15, and

6) A5G101 DGRWHRVAVIMG (Asp-Gly-Arg-Trp-His-Arg-Val-Ala-Val-Ile-Met-Gly) SEQ ID NO 18.

These laminin globular domain-derived peptides, such as the six just above from Sequence Group B, can be synthesized using L- or D-amino acids.

Once the above peptides are made, their D-amino acid forms and their parent L-amino acid forms may advantageously be assayed *in vitro* for amyloid inhibitory activity as described below. Those that are found to be efficacious are analyzed further in a number of different *in vitro* assays such as, to determine their binding affinity to Aβ, their ability to inhibit Aβ-Aβ self interactions (using a solid phase immunoassay), their effects on disruption/disassembly of β-pleated sheet (using Thioflavin T fluorometry, Congo Red binding assay, and circular dichroism spectroscopy), and their ability to inhibit Aβ fibril formation (by electron microscopy). Peptides that are active are further tested in cell culture for cellular toxicity, and for their potential to inhibit Aβ-induced neurotoxicity. If incorporation of tyrosine is found to reduce amyloid inhibitory activity, this step can be stepped by using a radio labeled D-amino acid as one of the reagents during synthesis to enhance bio-stability.

Active peptides can be linked to polyamine (putrescine, spermidine, or spermine) at the carboxy-terminal ends (Poduslo and Curran, J. Neurochem. 67:734-741, 1996) using the following procedure, as an example. Briefly, 2 ml of 0.4 M polyamine (putrescine, spermidine, or spermine), pH 4.7 (adjusted with HCl) is used to dissolve 1 mg of peptide. To this, 0.2 g of water-soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiamide is added. The reaction is stirred for 4 hours at room temperature and maintained at pH 4.7. The solution is purified by preparative HPLC, using instruments known to those skilled in the art, such as a HP 1100 series with diode array detector, with Vydac C18 column (21 x 250 mm) using 15%-40 % acetonitrile gradient over 80 minutes at a flow rate of 5 ml / min. Peptide peaks are pooled and lyophilized for further analysis. Some of the peptides ((those containing glutamate (E) or aspartate (D)) have a variable number of polyamines attached to them and thus are pooled separately from those containing one polyamine. The number of polyamines is determined based on the molecular weight increase from the parent peptide as determined, using instruments known to those skilled in the art, such as a Sciex API IIIE triple quadruple ion spray mass spectrometer. The polyamine-linked forms of peptides can also be assayed for amyloid-inhibitory activity

(described below). Further truncation of peptides can be performed in a similar manner depending on the potency of the resulting 7 amino acid peptide-analogs. Those that are determined to be efficacious are synthesized in labeled forms either by iodination of the tyrosine residues, or during synthesis using radiolabeled amino acids. The bio-stability of polyamine linkages can also be determined using ^{14}C labeled polyamine available from Sigma (Sigma Chem. Co. St Louis, MO, U.S.A.).

For the radio-iodination of peptide's tyrosine residues, the following procedure is used. Briefly, 0.5 mg of lyophilized peptides in a microcentrifuge tube is dissolved in 200µl of 0.5M phosphate buffer (pH 7.4), and Na ^{125}I solution (2-10µl; 0.2-1.0 mCi; ICN) is added. The iodination reaction is initiated by the addition of IodoBeads (Pierce, Rockford, Il). The tubes will be capped and left at room temperature. After 15 minutes, the reactions are stopped by removing the Iodo-Beads. The ^{125}I-labeled peptides are then applied to 1 gm of C18 sorbent (Varian Bond ElutÒSPE column, Walnut Creek, CA) and washed with 10 volumes of water containing 0.1% (w/v) cold iodine. Labeled peptides are then be eluted with 3 volumes of 50% (v/v) acetonitrile water, and the radioactivity is determined using instruments known to those skilled in the art, such as a MicroBeta TRILUX liquid Scintillation and luminescence counter (Wallac, Turku, Finland), and the radiolabeled peptides are lyophilized.

Example 6

In Vitro Testing to Determine Efficacy of Laminin Globular Domain-Derived Peptides as Aβ Amyloid Inhibitory Agents

The following are *in vitro* screening assays which are examples of testing procedures to determine the efficacy of L- and D-laminin globular domain derived peptides and analogs, as potential Aβ amyloid inhibitory agents.

Thioflavin T Fluorometry Assays:

Inhibition of Aβ fibril formation: Various peptides synthesized as outlined above can be tested for potential Aβ amyloid inhibitory activity using *in vitro* assays. Thioflavin T fluorometry, which measures the amount of amyloid fibrils formed (LeVine III, Protein Sci. 2:404-410, 1993; Amyloid: Int. J. Exp. Clin. Invest. 2:1-6, 1995; Naiki and Nakakuki, Lab. Invest., 74:374-383, 1996; Castillo et al, J. Neurochem. 69:2452-2465, 1997) can first be used to identify synthetic peptides capable of inhibiting Aβ 1-40 amyloid fibril formation. For these studies, 25 µM of Aβ 1-40 (Bachem Inc) is incubated in

microcentrifuge tubes at 37°C for 1 week (in triplicate), either alone, or in the presence of 25 μM, 50 μM or 250μM of parent peptides or peptide-analogs (at Aβ:peptide molar ratios of 1:1; 1:2 and 1:10 - or alternatively Aβ:peptide weight/weight ratios of 1:1, 1:0.1, 1:0.01, 1:0.001) in 150 mM Tris HCl, 10 mM NaCl, pH 7.0 (TBS). 50μl aliquots are taken for analysis at 1 hour, 1 day, 3 days and 1 week and added to 1.2 ml of 100 μM Thioflavin T and 50 mM NaPO4 (pH 6.0), respectively. Fluorescence emission at 480 nm is measured on a Turner model 450 fluorometer at an excitation wavelength of 450 nm. For each determination, the fluorometer is calibrated by zeroing in the presence of the Thioflavin T reagent alone, and by setting the 50 ng/ml riboflavin (Sigma) in the Thioflavin T reagent to 1800 fluorescence units. All fluorescence determinations are based on these references and any fluorescence given off by peptides in the presence of the Thioflavin T reagent is always subtracted from all pertinent readings. Our experience indicates that Thioflavin T does not give off any false fluorescence in the presence of laminin-derived peptides, nor do these peptides cause any quenching problems. Previous studies have also indicated that increasing concentrations of fibrillar Aβ gives a proportional increase in fluorescence in the presence of 100 μM Thioflavin T, ruling out the presence of any disproportionate inner filter effects at this Thioflavin T concentration (Castillo et al J. Neurochem. 69:2452-2465, 1997).

Disruption/Disassembly of Pre-formed Aβ Amyloid Fibrils: One can also determine the dose-dependent ability of laminin globular domain-derived peptides (and peptide analogs) to disrupt/disassemble preformed Aβ 1-40 and 1-42 fibrils. In these studies the peptides identified as inhibitors of Aβ 1-40 amyloid fibril formation (as described above) are used. For studies involving fibrillar Aβ 1-40, 1 mg of Aβ 1-40 (Bachem Inc) is dissolved in 1.0 ml of double distilled water (1mg/ml solution) and then incubated at 37°C for 1 week to cause abundant fibril formation. Aβ 1-42, which is already fibrillar, does not require pre-incubation at 37°C (as with Aβ 1-40), and is utilized immediately. For all of these studies, 25 μM of fibrillar amyloid (Aβ 1-40 or Aβ 1-42 is incubated in the presence of 150 mM Tris-HCl, 10 mM NaCl (pH 7.0), in the presence or absence of 25μM, 50μM, 125μM and 250 μM of the various parent peptides or peptide-analogs previously synthesized, giving approximate Aβ:peptide molar ratios of 1:1, 1:2, 1:5, 1:10. All samples, including controls (i.e. Aβ only, blank only or test -compound only) are tested in triplicate. Following an overnight, 3-day or 7-day incubation at 37°C, 50 μl aliquots are added to

1.2ml of 100μM Thioflavin T (Sigma) in 50mM NaPO4 (pH 6.0) for fluorometry readings as described above.

Statistical Analysis: For the fibril formation/disruption assays as described above, comparisons of Aβ 1-40 or Aβ 1-42 in the presence or absence of peptides is based on paired Student's t tests with data shown as mean +/-S.E., or ANOVA, depending on the particular study. Significance is reported at the 95% ($p<0.05$), 99% ($p<0.01$), and 99.9% ($p<0.001$) confidence levels.

Congo red Staining Assays: Aliquots (5μl) from the incubation assays as described above are also be analyzed by air-drying aliquots on gelatin-coated slides, followed by Congo red staining (Puchtler et al, J. Histochem. Cytochem. 10:355-364,1962). This technique has been effective in providing corroborating evidence of potential Aβ amyloid fibril inhibitors. A decrease in Congo red staining (i.e. red/green birefringence as viewed under polarized light) of fibrillar Aβ amyloid in the presence of peptides will confirm that a disruption/disassembly of amyloid fibril architecture has taken place. Further analysis of the most potent peptides identified at the light microscopic level will also analyzed by negative stain electron microscopy as described below.

Negative Stain Electron Microscopy: Laminin globular domain-derived peptides (or peptide analogs) able to inhibit Aβ 1-40 fibril formation, and disrupter/disassemble pre-formed Aβ 1-42 fibrils, as determined by Thioflavin T fluorometry and Congo red staining assays, (as described above), can be confirmed by negative stain electron microscopy. For confirmation of inhibition of Aβ 1-40 fibril formation, laminin globular domain-derived peptides (or peptide analogs) are incubated with 50μM of freshly solubilized Aβ 1-40 (Bachem) at Aβ:peptide molar ratios of 1:1, 1:2 and 1:10, for increasing times (i.e. 0 hours, 1 day, 3 days and 7 days) to observe any time-dependent and dose-dependent inhibition of Aβ 1-40 fibril formation. Comparisons are made to Aβ 1-40 only. Negatively stained Aβ fibrils are prepared by floating pioloform, carbon-coated grids on peptide solutions (200μg/ml of Aβ 1-40) in the presence of absence of various concentrations of laminin globular domain-derived peptides (as described above). To control for pH changes, peptides are dissolved in buffered solutions of 20 mM glycine (for pH 2 to 3 and pH 9 to 10) or 20 mM Tris-HCl (for pH 6 to 8). After the grids are blotted and air-dried, the samples are stained with either 2% (w/v) uranyl acetate or 1% (w/v) phosphotungstic acid and visualized, and photographed, with instruments known to those skilled in the art,

such as a Phillips CM-10 electron microscope, using 80kv accelerating voltage. The ability of peptides to disrupt the structure of amyloid fibrils can be qualitatively determined. In another study, negative stain electron microscopy can be utilized to confirm which laminin globular domain-derived peptides (or peptide analogs) are effective in disruption/ disassembly of pre-formed Aβ 1-42 fibrils. For these studies, 50μM of fibrillized Aβ 1-42 (prepared fresh) is incubated with laminin globular domain-derived peptides (or peptide analogs) at Aβ:peptide molar ratios of 1:1, 1:2 and 1:10 at 37°C for 7 days. Aliquots are taken at 0, 1, 3, and 7 days of incubation for analysis by negative stain electron microscopy as described above. Inhibitors/ disruptors of Aβ fibrillogenesis are identified by their ability to form amorphous non-fibrillar material. High magnification measurements (*i.e.* 100,000X) of Aβ amyloid fibrils (fibril diameter usually 7-10 nm) are compared to materials observed at 7 days following incubation with different peptides (as described above).

Example 7

Further In Vitro Testing to Determine Efficacy of Laminin Globular Domain-Derived Peptides as Aβ Amyloid Inhibitory Agents

The following are further *in vitro* screening assays which are examples of testing procedures to determine the efficacy of the D-forms of laminin globular domain derived peptides and analogs as potential Aβ amyloid inhibitory agents.

Figures 12a-f are laminin peptide sequences for the following peptides that are the subject of these discussions and of figures 13-26 herein. In the following tables and discussion, L is the L-form of the peptide, D (or D-) is used to denote, or as a prefix for, the D-form of the peptide (as distinguished from the L-form of the same peptide), D-R (or D-R-) is used to denote, or as a prefix for, the D-R-form of a D-form peptide, where the D-R-form is the reverse form of the D-peptide. Through the remainder of peptide efficacy discussion herein, peptides may be referred to either in the standard positional nomenclature (such as A5G101), with or without a D- or L- prefix (such as D-A5G101), or as the peptide's corresponding D-form peptide (DP) specimen number (such as DP6), or as the peptide's corresponding L-form peptide (LP) specimen number (such as LP24), or as the reverse D-form peptide's corresponding (DP) specimen number (such as DP18) and/or its standard reverse positional nomenclature (such as D-R-A5G101), or any combination of these.

Peptide designation	D	D-R	L
AG73	DP1	DP13	LP19
A13	DP2	DP14	LP20
HA3G82	DP3	DP15	LP21
A4G82	DP4	DP16	LP22
A5G81	DP5	DP17	LP23
A5G101	DP6	DP18	LP24

LP25 herein is synonymous with iAβ5. In addition 6 new 12-13 mer D-form peptides are assessed.

Peptide	D
HA3G47	DP7
HA3G58	DP8
HA3G74	DP9
HA3G83	DP10
A5G82	DP11
A5G109	DP12

A5G81 (DP5) also has six 7 mer truncations labeled DP26-31; A5G101 (DP6) has six 7 mer truncations labeled DP32-37; A4G82 (DP4) has six 7 mer truncations labeled DP38-38; and rHA3G76 (DP15, reverse DP3) has six 7 mer truncations labeled DP44-49.

Dose-Dependent Disassembly/Disruption of Pre-formed Aβ 1-42 Fibrils by Selected Laminin Globular Domain Derived Peptides

Thioflavin T fluorometry (ThioT), Congo Red (CR) and circular dichroism (CD) spectroscopy methods (see Figures 13-26) were used to assess the potential disruption ability of laminin globular domain derived peptides (12-13 mers) on pre-formed Aβ 1-42 fibrils (an important therapeutic target for Alzheimer's disease and other beta amyloid diseases). Testing of the top 6 laminin derived-peptides (including AG73, A13, HA3G76, A4G82, A5G81 and A5G101) and comparisons to the potential inhibitory activity of iAβ5 were determined. By Thioflavin T fluorometry, the results demonstrated that many of these laminin globular domain derived-peptides showed significant inhibition compared

to iAβ5 (LP25). Additionally, circular dichroism spectroscopy studies (Figures 17-26) were implemented to confirm the results obtained by Thioflavin T fluorometry.

Circular dichroism (CD) spectroscopy is an effective means to assess whether one mechanism of action involves the test compound's ability to disrupt the β-pleated sheet secondary structure present in Aβ fibrils. In on study, the typical pattern of a predominant β-pleated sheet structure (with a minima at 218nm) was observed with Aβ 1-42 fibrils following 3 days of incubation (see Figures 17a through 26a). No observable effect on disruption of β-sheet in Aβ 1-42 fibrils was found for iAβ5 at an Aβ42:iAβ5 molar ratio of 1:5. In contrast, for example, laminin derived-peptide DP1 AG73, showed a marked disruption of β-sheet structure as observed in a decrease in the minima band at 218nm. A similar disruption of Aβ 1-42 fibrils was observed with laminin derived-peptide DP5 A5G81, and to a lesser extent when using laminin derived peptide DP4 A4G82. Other favorably acting peptides include DP3, 6-7, 9-10, 12-13 and 15-18. Similar observations were obtained using fourier transform infrared (FTIR) spectroscopy, wherein it was observed that the indicated laminin derived peptides also significantly disrupted the β-sheet of Aβ 1-42 fibrils, and iAβ5 had no observable effect. These studies indicate that the selected 12-13 mer laminin derived peptides (in D-amino acid form) are effective as Aβ fibril disrupters and that one potential mechanism of action is disruption of β-sheet secondary structure characteristic of Aβ amyloid fibrils.

Synthesis of D-form Laminin Globular Domain-Derived Peptides

We have now synthesized the D-form of the 6 parent 12-13 amino acid peptides discussed above as Sequence Group B (all L-forms) as showing superior Aβ amyloid inhibitory activity. Since we earlier tested the L-form of each of the 6 laminin derived peptides, we chose to synthesize the D-amino acid form of the same 6 laminin derived peptides. Those D-form amino acids synthesized are: DP1) AG73 (RKRLQVQLSIRT), DP2) A13 (RQVFQVAYIIIKA), DP3) HA3G76 (YLSKGRLVFALG), DP4) A4G82 (TLFLAHGRLVFM), DP5) A5G81 (AGQWHRVSVRWG), and DP6) A5G101 (DGRWHRVAVIGM). The D amino acid form of these peptides is believed to offer some therapeutic advantage over L-form peptides, since the D amino acid peptides are known to be more resistant to *in vivo* protease degradation. In addition, the reverse sequences (DP13-18) of all 6 D-form peptides described above were also synthesized to determine if reversing the sequence alters potential Aβ amyloid inhibitory activity. Lastly, a group of

another 6 D amino acid 12-13 mer peptides (DP7-12) were also synthesized, and these represents 6 additional laminin derived peptides (also already tested in L amino acid form) that were only somewhat less effective than the first 6 peptides described above, still maintaining >25% Aβ fibril disrupting ability.

The six peptides from Sequence Group B, in either L- or D-form can be truncated into shorter 5 to 7 L- or D-amino acid peptides (for example) with or without tyrosine at the C-terminal end.

For example, representative DP1 D-AG73 peptide truncations (the resulting 7 L- or D-amino acid peptides synthesized and tested for amyloid inhibitory activity) are RKRLQVQ(Y), KRLQVQL(Y), RLQVQLS(Y), LQVQLSI(Y), QVQLSIR(Y) and, VQLSIRT(Y).

For example, for DP2 D-A13 peptide truncation, a resulting (7 L- or D-amino acid) peptides synthesized and tested for amyloid inhibitory activity is RQVFQUA, QVFQUAY, VFQUAYI, FQUAYII, QUAYIII, UAYIIIK, and AYIIIKA.

For example, for DP3 D-HA3G76 peptide truncation, a resulting (7 L- or D-amino acid) peptides synthesized and tested for amyloid inhibitory activity is YLSKGRL(Y), LSKGRLV(Y), SKGRLVF(Y), KGRLVFA(Y), GRLVFAL(Y), and RLVFALG(Y).

For example, for DP4 D-A4G82 peptide truncation, a resulting (7 L- or D-amino acid) peptides synthesized and tested for amyloid inhibitory activity is DP38 TLFLAHG(Y), DP39 LFLAHGR(Y), DP40 FLAHGRL(Y), DP41 LAHGRLV(Y), DP42 AHGRLVF(Y), and DP43 HGRLVFM(Y).

For example, for DP5 D-A5G81 peptide truncation, a resulting (7 L- or D-amino acid) peptides synthesized and tested for amyloid inhibitory activity is DP 26 AGQWHRV(Y), DP27 GQWHRVS(Y), DP28 QWHRVSV(Y), DP29 WHRVSVR(Y), DP30 HRVSVRW(Y), and DP31 RVSVRWG(Y).

For example, for DP6 D-A5G101peptide truncation, a resulting (7 L- or D-amino acid) peptides synthesized and tested for amyloid inhibitory is DP 32 DGRWHRV(Y), DP33 GRWHRVA(Y), DP34 RWHRVAV(Y), DP35 WHRVAVI(Y), DP36 HRVAVIM(Y), and DP37 RVAVIMG(Y).

In addition, for DP15 D-R-HA3G76 peptide truncation, a resulting (7 L- or D-amino acid) peptides synthesized and tested for amyloid inhibitory activity is DP44

GLAFVLR(Y), DP45 LAFVLRG(Y), DP46 AFVLRGK(Y), DP47 FVLRGKS(Y), DP48 VLRGKSL(Y), and DP49 LRGKSLY(Y).

As before with the above discussed 12-13 mer peptides, once the above peptides are made, their D-amino acid forms and their parent L-amino acid forms, along with the truncated 7 L- or D-amino acid peptides as described above, may advantageously be assayed *in vitro* for amyloid inhibitory activity as described herein. Those that are found to be efficacious are analyzed further in a number of different *in vitro* assays such as, to determine their binding affinity to Aβ, their ability to inhibit Aβ-Aβ self interactions (using a solid phase immunoassay), their effects on disruption/disassembly of β-pleated sheet (using circular dichroism spectroscopy), and their ability to inhibit Aβ fibril formation (by electron microscopy). Peptides that are active are further tested in cell culture for cellular toxicity, and for their potential to inhibit Aβ-induced neurotoxicity. If incorporation of tyrosine is found to reduce amyloid inhibitory activity, this step can be stepped by using a radio labeled D-amino acid as one of the reagents during synthesis to enhance bio-stability.

Thioflavin T Fluorometry Assay

A first set then of 12 synthesized six D-form peptides based upon the top performing six L-form peptides such as A13 discussed above, and respective reverse sequences of the six D-form peptides, was subjected to a battery of tests for potential ability of each tested peptide to disrupt/disassemble Aβ 1-42 fibrils as described above. Twelve laminin-peptides D-AG73 DP1, D-A13 DP2, D-HA3G82 DP3, D-A4G82 DP4, D-A5G81 DP5, D-A5G101 DP6, D-R-AG73 DP13, D-R-A13 DP14, D-R-HA3G82 DP15, D-R-A4G82 DP16, D-R-A5G81 DP17, and D-R-A5G101 DP18 were tested.

Using a Thioflavin T fluorometry assay of these 12 laminin-derived peptides, the D-form of laminin derived-peptide A13 (as described above) DP2 and its reverse D-form DP14 respectively, were most effective. In one study shown in Figure 13, 25μM Aβ 1-42 was incubated with decreasing concentrations of test peptides (at an Aβ:peptide wt/wt ratio of 1:1, 1:0.1, 1:0.01 and 1:0.001) for 3 days. The D-form reverse peptide of A13, D-R-A13 or DP14, was found to disrupt Aβ 1-42 fibrils by 79.1% when incubated at a 1:1 wt/wt ratio, and by 31.2% when incubated at a 1:0.1 wt/wt ratio. In comparison, in this particular study iAβ5, LP25, was found not to significantly disrupt Aβ 1-42 fibrils under the same conditions. The order of 5 of the most effective laminin derived peptides to

disrupt Aβ 1-42 fibrils at a 1:1 wt/wt ratio as determined by Thioflavin T fluorometry is D-R-A13 DP14 (79.1%)> D-R-A4G82 DP16 (36%) >D-A5G101 D6 and D-R-AG73 DP13 (both 34%) > D-A5G81 DP5 (32%).

A second set of six synthesized D-form peptides, D-HA3G47 DP7, D-HA3G58 DP8, D-HA3G74 DP9, D-HA3G83 DP10, D-A5G82 DP11, and D-A5G109 DP12, was also subjected to the same test for their potential to disrupt/disassemble Aβ 1-42 fibrils as described above. Figure 15 shows results of the same Thioflavin T fluorometry assay discussed just above, where 25μM Aβ 1-42 was incubated with decreasing concentrations of test peptides (at an Aβ:peptide wt/wt ratio of 1:1, 1:0.1, 1:0.01 and 1:0.001) for 3 days. The D-form peptide of HA3G58 DP8 was found to disrupt Aβ 1-42 fibrils by ~ 75% when incubated at a 1:1 wt/wt ratio, and by ~40% when incubated at a 1:0.1 wt/wt ratio. In comparison, iAβ5, LP25, was found not to significantly disrupt Aβ 1-42 fibrils under the same conditions. The order of 4 of the most effective laminin derived peptides to disrupt Aβ 1-42 fibrils at a 1:1 wt/wt ratio as determined by Thioflavin T fluorometry is D (form) HA3G58 DP8 > D-A4G82 DP4 = D-A5G101 D6 = D-HA3G74 DP9 (all ~50%).

Congo Red Binding Assay

Using a Congo red (CR) binding assay, see Figure 14, the ability of the 12 synthesized peptides, D-AG73 DP1, D-A13 DP2, D-HA3G82 DP3, D-A4G82 DP4, D-A5G81 DP5, D-A5G101 DP6, D-R-AG73 DP13, D-R-A13 DP14, D-R-HA3G82 DP15, D-R-A4G82 DP16, D-R-A5G81 DP17, and D-R-A5G101 DP18, to inhibit the binding of Congo red to Aβ 1-42 fibrils was also assessed. This assay is another measure of a test peptide's ability to disrupt Aβ fibrils. Superior laminin derived peptide inhibition of CR binding to Aβ 42 fibrils was again found to be D-R-A13, DP14, which caused a 100% inhibition when used at an Aβ:peptide wt/wt ratio of 1:1, and a 75.1 % inhibition when used at a 1:0.1 wt/wt ratio. In comparison, LP25 iAβ5 only inhibited Congo red binding by 39.6% at a 1:1 wt/wt ratio, and by 31.8% when used at a 1:0.01 wt/wt ratio. The order of 5 of the most effective laminin derived peptides to inhibit Congo red binding to Aβ 42 fibrils at a 1:1 wt/wt ratio as determined by this assay is D-R-A13 DP14 (100%)> D-A13 DP2 (99%) > D-R-A4G82 DP16 (94.8%) > D-A4G82 DP4 (55.0%) > D-A5G81 DP5 (54.5%). These results obtain notwithstanding that DP2 D-A13 and its reverse peptide DP14 D-R-A13 appear to contain substantial intrinsic β-sheet structure of their own, which is picked up in the ThioT assay.

Using a Congo red (CR) binding assay, see Figure 16, the ability of the same synthesized peptides, D-HA3G47 DP7, D-HA3G58 DP8, D-HA3G74 DP9, D-HA3G83 DP10, D-A5G82 DP11, and D-A5G109 DP12, as discussed with respect to Figure 15 to inhibit the binding of Congo red to Aβ 1-42 fibrils was also assessed. Superior laminin derived peptide inhibition of CR binding to Aβ 42 fibrils was found to be HA3G58 DP8 and HA3G83 DP10 , both of which caused a 100% inhibition when used at an Aβ:peptide wt/wt ratio of 1:1, and a 100% inhibition when used at a 1:0.1 wt/wt ratio. In comparison, LP25 iAβ5 did not significantly inhibit Congo red binding at a 1:1 wt/wt ratio. The order of 5 of the most effective laminin derived peptides to inhibit Congo red binding to Aβ 42 fibrils at a 1:1 wt/wt ratio as determined by this assay is D-HA3G58 DP8 and D-HA3G83 DP10 (both 100%) > D-R-A13 DP14 (~95%)> D-A5G82 DP11 (~90%) > D-A13 DP2 (~85%).

Circular Dichroism Spectroscopy

Circular dichroism (CD) spectroscopy/FTIR studies and negative stain electron microcopy studies were also done to confirm the potential disruptive/inhibitory effects of the laminin derived peptides as described above. In one CD study the effects of different laminin derived peptides on disruption of Aβ 1-42 fibrils was assessed following a 3-day incubation of test peptides at an Aβ:peptide wt/wt ratio of 1:2 (~1:6 Aβ:peptide molar ratio). The results shown in Figures 17-26 demonstrate that DP6 D-A5G101 and DP5 D-A5G81 were most effective in causing an almost complete disruption (i.e. ~95%) of Aβ 1-42 β-sheet structure. Also effective but to a lesser extent (50-60% disruption) were DP17 D-R-A5G81, DP16 D-R-A4G82 and DP14 D-R-A13. As these 3 latter peptides appear to contain intrinsic β-sheet structure of their own (especially DP14 D-R-A13), sometimes making the CD data difficult to interpret. On the other hand, both DP6 D-A5G101 and DP5 D-A5G81 peptides contain little intrinsic β-sheet structure themselves. For further data on amino acid sequences for respective DP numbers 1-18 and CD ellipticity loss, see Table 1 at the end of this specification.

Further Aspects and Utilizations of the Invention

Laminin-Derived Polypeptides

One therapeutic application of the present invention is to use laminin-derived polypeptides as potent inhibitors of Aβ amyloid formation, deposition, accumulation and/or persistence, in Alzheimer's disease, Down's syndrome and other amyloid disorders involving Aβ fibrillogenesis.

The polypeptide referred to above may be a natural polypeptide, a synthetic polypeptide or a recombinant polypeptide. The polypeptides, derivatives or analogs referred to herein may be a) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue and such substituted amino acid residue may or not be encoded by the genetic code, or b) in which one or more of the amino acid residues includes a substituent group, or c) one in which the mature polypeptide is fused with another compound, such as a compound used to increase the half-life of the polypeptide (for example, polylysine), or d) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such polypeptides, derivatives and analogs are deemed to be within the scope of the invention.

Protein conformation is an essential component of protein-protein, protein-substrate, protein-agonist, protein-antagonist interactions. Changes in the component amino acids of protein sequences can result in changes that have little or no effect on the resultant protein conformation. Conversely, changes in the peptide sequences can have effects on the protein conformation resulting in reduced or increased protein-protein interactions. Such changes and their effects are generally disclosed in Proteins: Structures and Molecular Properties by Thomas Creighton, W.H. Freeman and Company, New York, 1984 which is hereby incorporated by reference.

It will be appreciated by those skilled in the art that changes can be made to the disclosed laminin polypeptides, derivatives or analogs, that increase, decrease or otherwise have no effect on the binding of laminin or fragments thereof to Aβ amyloid. In addition, it will be appreciated by those skilled in the art that various post-translational modifications such as phosphorylation, glycosylation and the like, will alter the binding of laminin polypeptides, derivatives or analogs to Aβ amyloid.

The polypeptides of the present invention include the polypeptides described herein, including but not limited to AG73 (SEQ ID NO:1), C-16 (SEQ ID NO:2), A-13 (SEQ ID NO:3), HA3G47 (SEQ ID NO:4), HA3G58 (SEQ ID NO:5), HA3G67 (SEQ ID NO:6), HA3G74 (SEQ ID NO:7), HA3G76 (SEQ ID NO:8), HA3G79 (SEQ ID NO:9), HA3G83 (SEQ ID NO:10), A4G82 (SEQ ID NO:11), A5G15 (SEQ ID NO:12), A5G56 (SEQ ID NO:13), A5G80 (SEQ ID NO:14), A5G81 (SEQ ID NO:15), A5G82 (SEQ ID NO: 16), A5G84

(SEQ ID NO:17), A5G101 (SEQ ID NO:18), A5G109 (SEQ ID NO:19) (Sequence Group A), and fragments thereof, as well as polypeptides which preferably have at least a 70%, and more preferably a 90% identity, to the polypeptides described above. "% Identity" as used herein for peptides means the same amino acids in the same place. Thus a ten amino acid peptide that is identical to another ten amino acid peptide, except for one amino acid, is 90% identical. If a ten amino acid peptide has the same ten amino acids in the same number of each amino acid as another ten amino acid peptide, but two amino acids are juxtaposed with each other, then the two amino acids have an 80% identity with each other, and so forth.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture), or from a virus (such as with the use of phage display techniques known to those skilled in the art). Depending upon the host employed in a recombinant procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

Chemical polypeptide synthesis is a rapidly evolving area in the art, and methods of solid phase polypeptide synthesis are well-described in the following references, hereby entirely incorporated by reference (Merrifield, J.Amer.Chem.Soc. 85:2149-2154, 1963; Merrifield, Science 232:341-347, 1986; Fields, Int.J.Polypeptide Prot. Res. 35, 161, 1990).

Recombinant production of laminin polypeptides can be accomplished according to known method steps. Standard reference works setting forth the general principles of recombinant DNA technology include Watson, Molecular Biology of the Gene, Volumes I and II, The Benjamin/Cummings Publishing Company Inc., publisher, Menlo Park, Calif. 1987; Ausubel et al, eds., Current Protocols in Molecular Biology, Wiley Interscience, publisher, New York, N.Y. 1987; 1992; and Sambrook et al, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, publisher, Cold Spring Harbor, N.Y. 1989, the entire contents of which references are herein incorporated by reference.

The polypeptides of the present invention may also be utilized as research reagents and materials for discovery of treatments and diagnostics for human diseases.

Antibodies

Antibodies generated against the polypeptides corresponding to specific sequences recognizing the laminin fragments of the present invention which bind Aβ can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptides from tissue expressing that polypeptide. Preferred embodiments include, but are not limited to, Sequence Group A, and fragments thereof, as well as polypeptides which have at least 70% identity and more preferably a 90% identity to the polypeptides described above.

The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies, chimeric antibodies, anti-idiotypic antibodies to antibodies specific for laminin-derived protein fragments or polypeptides of the present invention.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen.

A monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which population contains substantially similar epitope binding sites. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, Nature 256:495-497, 1975), the trioma technique, the human B-cell hybridoma technique (Kozbor et al, Immunology Today 4:72, 1983), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp.77-96, 1985). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof.

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, which are primarily used to reduce immunogenicity in application and to increase yields in production. Chimeric antibodies and methods for their production are known in the art (ex. Cabilly

et al, Proc. Natl. Acad. Sci. U.S.A 81:3273-3277, 1984; Harlow and Lane: Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory 1988).

An anti-idiotypic antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An anti-idiotypic antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the monoclonal antibody with the monoclonal antibody to which an anti-idiotypic antibody is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-idiotypic antibody). See, for example, U.S. Patent No. 4,699,880, which is herein incorporated by reference.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab")_2$, which are capable of binding antigen. Fab and $F(ab")_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al, J. Nucl. Med. 24:316-325, 1983).

The antibodies or fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect laminin-derived fragments in a sample or to detect presence of cells which express a laminin polypeptide of the present invention. This can be accomplished by immunofluorescence techniques employing a flourescently labeled antibody coupled with light microscopic, flow cytometric or fluorometric detection.

One of the ways in which a laminin fragment antibody can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA). This enzyme, in turn, when later exposed to an appropriate substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric, or by visual means. Enzymes which can be used detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colormetric methods which employ a chromogenic substrate for the enzyme. Detection can be

accomplished by colormetric methods which employ a chromogenic substrate for the enzyme. Detection can also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate with similarly prepared standards (see Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory 1988; Ausubel et al, eds., Current Protocols in Molecular Biology, Wiley Interscience, N.Y. 1987, 1992).

Detection may be accomplished using any of a variety of other immunoassays. For example, by radiolabeling of the antibodies or antibody fragments, it is possible to detect R-PTPase through the use of a radioimmunoassay (RIA). A good description of RIA may be found in Laboratory Techniques and Biochemistry in Molecular Biology, by Work et al, North Holland Publishing Company, NY (1978) with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, incorporated entirely by reference herein. The radioactive isotope can be detected by such means as the use of a gamma-counter, a scintillation counter or by autoradiography.

It is also possible to label a laminin fragment polypeptide antibody with a fluorescent compound. When the flourescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine, commercially available, e.g., from Molecular Probes, Inc. (Eugene, Oregon, U.S.A.).

The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}EU, or other of the lanthanide series. These metals can be attached to the antibody using such metal groups as diethylenetriamine pentaacetic acid (EDTA).

The antibody can also be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt, and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent

reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, lucifers and aequorin.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for in situ detection of a laminin fragment of the present invention. In situ detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of a laminin fragment polypeptide but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

Antibodies against laminin fragments and/or laminin-derived polypeptides which interact with Aβ or other amyloid proteins, or derivatives thereof are also disclosed herein. These antibodies can be used for a number of important diagnostic and/or therapeutic applications as described herein. In one aspect of the invention, polyclonal and/or monoclonal antibodies made against laminin fragments and/or laminin-derived polypeptides which bind Aβ or other amyloid proteins, may be utilized for Western blot analysis (using standard Western blotting techniques knowledgeable to those skilled in the art) to detect the presence of amyloid protein-binding laminin fragments or amyloid protein-binding laminin polypeptides in human tissues and in tissues of other species. Western blot analysis can also be used to determine the apparent size of each amyloid protein-binding laminin fragment. In addition, Western blotting following by scanning densitometry (known to those skilled in the art) can be used to quantitate and compare levels of each of the laminin fragments or polypeptides in tissue samples, biological fluids or biopsies obtained from individuals with specific diseases (such as the amyloid diseases) in comparison to tissue samples, biological fluids or biopsies obtained from normal individuals or controls. Biological fluids, include, but are not limited to, blood, plasma, serum, cerebrospinal fluid, sputum, saliva, urine and stool.

In yet another aspect of the invention, polyclonal and/or monoclonal antibodies made against laminin fragments and/or laminin-derived peptides which bind Aβ or other amyloid proteins, can be utilized for immunoprecipitation studies (using standard immunoprecipitation techniques known to one skilled in the art) to detect laminin fragments and/or laminin-derived peptides which bind Aβ or other amyloid proteins, in tissues, cells and/or biological fluids. Use of the laminin fragment and/or laminin-derived peptide antibodies for immunoprecipitation studies can also be quantitated to determine relative levels of laminin fragments and/or laminin-derived peptides which interact with Aβ or other amyloid proteins, in tissues, cells and/or biological fluids. Quantitative immunoprecipitation can be used to compare levels of laminin fragments and/or laminin amyloid protein-binding peptides in tissue samples, biological fluids or biopsies obtained from individuals with specific diseases (such as the amyloid diseases) in comparison to tissue samples, biological fluids or biopsies obtained from normal individuals or controls.

Therapeutic Applications

Yet another aspect of the present invention is to make use of laminin fragments and/or laminin-derived polypeptides as amyloid inhibitory therapeutic agents. The laminin-derived peptide sequences or fragments can be synthesized utilizing standard techniques (*i.e.* using an automated synthesizer). Laminin fragments and/or laminin-derived polypeptides which bind Aβ or other amyloid proteins, can be used as potential blocking therapeutics for the interaction of laminin in a number of biological processes and diseases (such as in the amyloid diseases described above). In a preferred embodiment, specific laminin-derived polypeptides may be used to aid in the inhibition of Aβ amyloid formation, deposition, accumulation, and /or persistence in a given patient. Likewise, in another preferred embodiment anti-idiotypic antibodies made against laminin fragments and/or laminin-derived polypeptides (as described above) may be given to a human patient as potential blocking antibodies to disrupt continued Aβ amyloid formation, deposition, accumulation and/or persistence in the given patient.

Preparations of laminin-derived polypeptides for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain axillary agents or excipients which are known in the art. Pharmaceutical compositions such as tablets, pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, syrups, tea

bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, sterile packaged powders, can be prepared according to routine methods and are known in the art.

In yet another aspect of the invention, laminin fragments and/or laminin-derived polypeptides may be used as an effective therapy to block Aβ amyloid formation, deposition, accumulation and/or persistence as observed in the amyloid diseases. For example, the invention includes a pharmaceutical composition for use in the treatment of amyloidoses comprising a pharmaceutically effective amount of a laminin fragment and/or laminin-derived polypeptide anti-idiotypic antibody and a pharmaceutically acceptable carrier. The compositions may contain the laminin fragments and/or laminin-derived polypeptide anti-idiotypic antibody, either unmodified, conjugated to a potentially therapeutic compound, conjugated to a second protein or protein portion or in a recombinant form (i.e. chimeric or bispecific laminin fragment and/or laminin polypeptide antibody). The compositions may additionally include other antibodies or conjugates. The antibody compositions of the invention can be administered using conventional modes of administration including, but not limited to, topical, intravenous, intra-arterial, intraperitoneal, oral, intralymphatic, intramuscular or intralumbar. Intravenous administration is preferred. The compositions of the invention can be a variety of dosage forms, with the preferred form depending upon the mode of administration and the therapeutic application. Optimal dosage and modes of administration for an individual patient can readily be determined by conventional protocols.

Laminin-derived protein fragments, and laminin-derived polypeptides, or antibodies of the present invention may be administered by any means that achieve their intended purpose, for example, to treat laminin involved pathologies, such as Alzheimer's disease and other amyloid diseases, or other related pathologies, using a laminin-derived polypeptide described herein, in the form of a pharmaceutical composition.

For example, administration of such a composition may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal or buccal routes. Alternatively, or concurrently, administration may be by the oral route. Parenteral administration can be by bolus injection or by gradual perfusion over time.

A preferred mode of using a laminin-derived polypeptide, or antibody pharmaceutical composition of the present invention is by oral administration or intravenous application.

A typical regimen for preventing, suppressing or treating laminin-involved pathologies, such as Alzheimer's disease amyloidosis, comprises administration of an effective amount of laminin-derived polypeptides, administered over a period of one or several days, up to and including between one week and about 24 months.

It is understood that the dosage of the laminin-derived polypeptides of the present invention administered *in vivo* or *in vitro* will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation.

The total dose required for each treatment may be administered by multiple doses or in a single dose. A laminin-derived polypeptide may be administered alone or in conjunction with other therapeutics directed to laminin-involved pathologies, such as Alzheimer's disease or other Aβ amyloid diseases, as described herein.

Effective amounts of a laminin-derived polypeptide or composition, which may also include a laminin-fragment derived antibody, are about 0.01μμg to about 100mg/kg body weight, and preferably from about 10 μg to about 50 mg/kg body weight, such as 0.05, 0.07, 0.09, 0.1, 0.5, 0.7, 0.9., 1, 2, 5, 10, 20, 25, 30, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 mg/kg.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain axillary agents or excipients which are known in the art. Pharmaceutical compositions comprising at least one laminin-derived polypeptide, such as 1-10 or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 laminin-derived polypeptides, of the present invention may include all compositions wherein the laminin-derived polypeptide is contained in an amount effective to achieve its intended purpose. In addition to at least one laminin-derived polypeptide, a pharmaceutical composition may contain suitable pharmaceutically acceptable carriers, such as excipients, carriers and/or axillaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically.

Pharmaceutical compositions comprising at least one laminin-derived polypeptide or antibody may also include suitable solutions for administration intravenously, subcutaneously, dermally, orally, mucosally, rectally or may by injection or orally, and contain from about 0.01 to 99 percent, preferably about 20 to 75 percent of active component (i.e. polypeptide or antibody) together with the excipient. Pharmaceutical compositions for oral administration include pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, and syrups.

The laminin-derived protein fragments, and laminin-derived polypeptides for Alzheimer's disease and other central nervous system Aβ amyloidoses may be optimized to cross the blood-brain barrier. Methods of introductions include but are not limited to systemic administration, parenteral administration i.e., via an intraperitoneal, intravenous, perioral, subcutaneous, intramuscular, intraarterial, intradermal, intramuscular, intranasal, epidural and oral routes. In a preferred embodiment, laminin-derived protein fragments, and laminin-derived polypeptides may be directly administered to the cerebrospinal fluid by intraventricular injection. In a specific embodiment, it may be desirable to administer laminin-derived protein fragments, and laminin-derived polypeptides locally to the area or tissue in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, by injection, by infusion using a cannulae with osmotic pump, by means of a catheter, by means of a suppository, or by means of an implant.

In yet another embodiment laminin-derived protein fragments, and laminin-derived polypeptides may be delivered in a controlled release system, such as an osmotic pump. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i.e. the brain, thus requiring only a fraction of the systemic dose.

In yet another aspect of the present invention, peptidomimetic compounds modeled from laminin fragments and/or laminin-derived polypeptides identified as binding Aβ or other amyloid proteins, may serve as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other Aβ amyloidoses. Peptidomimetic modeling is implemented by standard procedures known to those skilled in the art.

In yet another aspect of the present invention, compounds that mimic the 3-dimensional Aβ binding site on laminin using computer modeling, may serve as potent inhibitors of Aβ amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other Aβ amyloidoses. Design and production of such compounds using computer modeling technologies is implemented by standard procedures known to those skilled in the art.

Recombinant DNA technology, including human gene therapy, has direct applicability to the laminin polypeptides, of this invention. One skilled in the art can take the peptide sequences disclosed herein and create corresponding nucleotide sequences that code for the corresponding peptide sequences. These sequences can be cloned into vectors such as retroviral vectors, and the like. These vectors can, in turn, be transfected into human cells such as hepatocytes or fibroblasts, and the like. Such transfected cells can be introduced into humans to treat amyloid diseases. Alternatively, the genes can be introduced into the patients directly. The basic techniques of recombinant DNA technology are known to those of ordinary skill in the art and are disclosed in Recombinant DNA Second Edition, Watson, et al., W.H. Freeman and Company, New York, 1992, which is hereby incorporated by reference.

Diagnostic Applications

Another aspect of the invention is to provide polyclonal and/or monoclonal antibodies against laminin fragments and/or laminin-derived polypeptides which bind Aβ or other amyloid proteins, which is utilized to specifically detect laminin fragments and/or laminin-derived peptides in human tissues and/or biological fluids. In one preferred embodiment, polyclonal or monoclonal antibodies made against a peptide portion or fragment of laminin, can be used to detect and quantify laminin fragments and/or laminin-derived polypeptides in human tissues and/or biological fluids. Polyclonal and/or monoclonal peptide antibodies can also be utilized to specifically detect laminin fragments and/or laminin-derived polypeptides in human tissues and/or biological fluids. In a preferred embodiment, a polyclonal or monoclonal antibody made specifically against a peptide portion or fragment of laminin fragments or polypeptides which bind Aβ (as described herein), can be used to detect and quantify these laminin fragments or polypeptides in human tissues and/or biological fluids. Other preferred embodiments include, but are not limited to, making polyclonal or monoclonal antibodies made

specifically against a peptide portion or fragment of any of the peptides of Sequence Group A, as well as polypeptides which have at least 70% identity and more preferably a 90% identity to the polypeptides described above. For detection of laminin fragments and/or laminin-derived polypeptides described above in human tissues, cells, and/or in cell culture, the polyclonal and/or monoclonal antibodies can be utilized using standard immunohistochemical and immunocytochemical techniques, known to one skilled in the art.

For detection and quantitation of laminin fragments and/or laminin-derived polypeptides in biological fluids, including cerebrospinal fluid, blood, plasma, serum, urine, sputum, and/or stool, various types of ELISA assays can be utilized, known to one skilled in the art. An antibody molecule of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or 'sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support or carrier, and a quantity of detectable labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

In a preferred embodiment, a 'sandwich" type of ELISA can be used. Using this preferred method a pilot study is first implemented to determine the quantity of binding of each laminin-fragment or polypeptide monoclonal antibody to microtiter wells. Once this is determined, aliquots (usually in 40 µl of TBS; pH 7.4) of the specific laminin-fragment or laminin polypeptide antibody are allowed to bind overnight to microtiter wells (Maxisorb C plate from Nunc) at 4°C. A series of blank wells not containing any laminin-fragment or laminin polypeptide specific monoclonal antibody are also utilized as controls. The next day, non-bound monoclonal antibody is shaken off the microtiter wells. All of the microtiter wells (including the blank wells) are then blocked by incubating for 2 hours with 300 µl of Tris-buffered saline containing 0.05% Tween-20 (TTBS) plus 2% bovine serum albumin, followed by 5 rinses with TTBS. 200 µl of cerebrospinal fluid, blood, plasma, serum, urine, sputum, and/or stool and/or any other type of biological sample is then diluted (to be determined empirically) in TTBS containing 2% bovine serum albumin and placed in wells (in triplicate) containing bound laminin-fragment or laminin-polypeptide antibody (or blank) and incubated for 2 hours at room temperature. The wells are then washed 5 times with TTBS. A second

biotinylated-monoclonal antibody against the same laminin-derived fragment or laminin polypeptide (but which is against a different epitope) is then added to each well (usually in 40 µl of TBS; pH 7.4) and allowed to bind for 2 hours at room temperature to any laminin-fragment or laminin polypeptide captured by the first antibody. Following incubation, the wells are washed 5 times with TTBS. Bound materials are then detected by incubating with 100 µl of peroxidase-avidin complex (1:250 dilution in TTBS with 0.1% BSA) for 1 hour on a rotary shaker. After 5 washes with TTBS, a substrate solution (100 µl, OPD-Sigma Fast from Sigma Chemical Co., St. Louis, MO, USA) is added and allowed to develop significant color (usually 8-10 minutes). The reaction is stopped with 50 µl of 4N sulfuric acid and read on a standard spectrophotometer at 490 nm. This ELISA can be utilized to determine differences in specific laminin fragments or polypeptides (and/or Aβ-binding laminin fragments or polypeptides) in biological fluids which can serve as a diagnostic marker to follow the progression in a live patient during the progression of disease (i.e. monitoring of Aβ amyloid disease as an example). In addition, quantitative changes in laminin fragments or laminin polypeptides can also serve as a prognostic indicator monitoring how a live patient will respond to treatment which targets an Aβ amyloid disease such as Alzheimer's disease. Such assays can be provided in a kit form.

A competition assay may also be employed wherein antibodies specific to laminin fragments and/or laminin-derived polypeptides are attached to a solid support and labeled laminin fragments and/or laminin-derived polypeptides and a sample derived from a host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to the quantity of laminin fragments and/or laminin-derived polypeptides in the sample. This standard technique is known to one skilled in the art.

Another object of the present invention is to use laminin fragments and/or laminin-derived polypeptides, in conjunction with laminin fragment and/or laminin-derived peptide antibodies, in an ELISA assay to detect potential laminin fragment and/or laminin-derived peptide autoantibodies in human biological fluids. Such a diagnostic assay may be produced in a kit form. In a preferred embodiment, peptides containing the sequences of laminin-derived fragments and laminin-derived polypeptides as in Sequence Group A, as well as polypeptides which have at least 70% identity and more preferably a 90% identity to the polypeptides described above, will be used to initially bind to microtiter wells in an ELISA plate.

A pilot study is first implemented to determine the quantity of binding of each laminin fragment or polypeptide to microtiter wells. Once this is determined, aliquots (usually 1-2μg in 40 μl of TBS; pH 7.4) of specific laminin fragment polypeptides (as described herein) are allowed to bind overnight to microtiter wells (Maxisorb C plate from Nunc) at 4°C. All the microtiter wells (including blank wells without the laminin fragment polypeptides) are blocked by incubating for 2 hours with 300 μl of Tris-buffered saline (pH 7.4) with 0.05% Tween-20 (TTBS), containing 2% albumin. This is followed by 5 rinses with TTBS. The patients" biological fluids (i.e., cerebrospinal fluid, blood, plasma, serum, sputum, urine, and/or stool) are then utilized and 200 μl are diluted (to be determined empirically) with TTBS containing 2% bovine serum albumin, and placed in microtiter wells (in triplicate) containing a specific laminin fragment polypeptide or blank wells (which do not contain peptide), and are incubated at 1.5 hours at room temperature.

Any autoantibodies present in the biological fluids against the laminin fragment or polypeptide will bind to the substrate bound laminin fragment polypeptide (or fragments thereof). The wells are then rinsed by washing 5 times with TTBS. 100 μl of biotinylated polyclonal goat anti-human IgG's (Sigma Chemical company, St. Louis, MO, USA), diluted 1:500 in TTBS with 0.1% bovine serum albumin, is then aliquoted into each well. Bound materials are detected by incubating with 100 μl of peroxidase-avidin complex (1:250 dilution in TTBS with 0.1% bovine serum albumin) for 1 hour on a rotary shaker. Following 5 washes with TTBS, substrate solution (100 μl, OPD-Sigma Fast from Sigma Chemical Company, St. Louis, MO, USA) is added and allowed to develop significant color (usually 8-10 minutes). The reaction is stopped with 50 μl of 4N sulfuric acid added to each well and read on a standard spectrophotometer at 490 nm.

This assay system can be utilized to not only detect the presence of autoantibodies against laminin fragments or polypeptides in biological fluids, but also to monitor the progression of disease by following elevation or diminution of laminin fragment or polypeptide autoantibody levels. It is believed that patients demonstrating excessive laminin fragment or polypeptide formation, deposition, accumulation and/or persistence as may be observed in the Aβ amyloid diseases, will also carry autoantibodies against the laminin fragments or laminin polypeptides in their biological fluids. Various ELISA assay systems, knowledgeable to those skilled in the art, can be used to accurately monitor the

degree of laminin fragments or polypeptides in biological fluids as a potential diagnostic indicator and prognostic marker for patients during the progression of disease (i.e. monitoring of an Aβ amyloid disease for example). Such assays can be provided in a kit form. In addition, quantitative changes in laminin fragment or polypeptide autoantibody levels can also serve as a prognostic indicator monitoring how a live patient will respond to treatment which targets an Aβ amyloid disease.

Other diagnostic methods utilizing the invention include diagnostic assays for measuring altered levels of laminin fragments and/or laminin-derived polypeptides in various tissues compared to normal control tissue samples. Assays used to detect levels of laminin fragments and/or laminin-derived polypeptides in a sample derived from a host are well-known to those skilled in the art and included radioimmunoassays, competitive-binding assays, Western blot analysis and preferably ELISA assays (as described above).

Yet another aspect of the present invention is to use the antibodies recognizing laminin fragments and/or laminin-derived polypeptides for labelings, for example, with a radionucleotide, for radioimaging or radioguided surgery, for *in vivo* diagnosis, and/or for in vitro diagnosis. In one preferred embodiment, radiolabeled peptides or antibodies made (by one skilled in the art) against laminin fragments and/or laminin-derived polypeptides may be used as minimally invasive techniques to locate laminin fragments and/or laminin-derived polypeptides, and concurrent Aβ amyloid deposits in a living patient. These same imaging techniques can then be used at regular intervals (i.e. every 6 months) to monitor the progression of the Aβ amyloid disease by following the specific levels of laminin fragments and/or laminin-derived polypeptides.

Yet another aspect of the present invention is to provide a method which can evaluate a compound's ability to alter (diminish or eliminate) the affinity of Aβ (as described herein) or amyloid precursor protein, to laminin-derived fragments or laminin-derived polypeptides. By providing a method of identifying compounds which affect the binding of Aβ amyloid protein, or amyloid precursor protein to such laminin-derived fragments or polypeptides, the present invention is also useful in identifying compounds which can prevent or impair such binding interactions. Thus, compounds can be identified which specifically affect an event linked with Aβ amyloid

formation, amyloid deposition, and/or amyloid persistence condition associated with Alzheimer's disease and other Aβ amyloid diseases as described herein.

According to one aspect of the invention, to identify for compounds which allow the interaction of Aβ†amyloid proteins or precursor proteins to laminin-derived fragments or laminin polypeptides, either Aβ†amyloid or laminin fragments or polypeptides are immobilized, and the other of the two is maintained as a free entity. The free entity is contacted with the immobilized entity in the presence of a test compound for a period of time sufficient to allow binding of the free entity to the immobilized entity, after which the unbound free entity is removed. Using antibodies that recognize the free entity, or other means to detect the presence of bound components, the amount of free entity bound to immobilized entity can be measured. By performing this assay in the presence of a series of known concentrations of test compound and, as a control, the complete absence of test compound, the effectiveness of the test compound to allow binding of free entity to immobilized entity can be determined and a quantitative determination of the effect of the test compound on the affinity of free entity to immobilized entity can be made. By comparing the binding affinity of the Aβ amyloid-laminin fragment or polypeptide complex in the presence of a test compound to the binding affinity of the amyloid-laminin fragment or polypeptide complex in the absence of a test compound, the ability of the test compound to modulate the binding can be determined. In the case in which the Aβ amyloid is immobilized, it is contacted with free laminin-derived fragments or polypeptides, in the presence of a series of concentrations of test compound. As a control, immobilized Aβ amyloid is contacted with free laminin-derived polypeptides, or fragments thereof in the absence of the test compound. Using a series of concentrations of laminin-derived polypeptides, the dissociation constant (K_d) or other indicators of binding affinity of amyloid-laminin fragment or polypeptide binding can be determined. In the assay, after the laminin-derived polypeptides or fragments thereof are placed in contact with the immobilized Aβ amyloid for a sufficient time to allow binding, the unbound laminin polypeptides are removed. Subsequently, the level of laminin fragment or polypeptide-Aβ amyloid binding can be observed. One method uses laminin-derived fragment or polypeptide antibodies, as described in the invention, to detect the amount of specific laminin fragments or polypeptides bound to the Aβ amyloid or the amount of free laminin fragments remaining in solution. This information is used to determine first

qualitatively whether or not the test compound can allow continued binding between laminin-derived fragments or polypeptides and Aβ amyloid. Secondly, the data collected from assays performed using a series of test compounds at various concentrations, can be used to measure quantitatively the binding affinity of the laminin fragment or polypeptide-Aβ amyloid complex and thereby determine the effect of the test compound on the affinity between laminin fragments or polypeptides and Aβ amyloid. Using this information, compounds can be identified which do not modulate the binding of specific laminin fragments or polypeptides to amyloid and thereby allow the laminin-fragments or polypeptides to reduce the Aβ amyloid formation, deposition, accumulation and/or persistence, and the subsequent development and persistence of Aβ amyloidosis.

Therefore a kit for practicing a method for identifying compounds useful which do not alter laminin-derived fragments or laminin-derived polypeptides to an immobilized Aβ amyloid protein, said kit comprising a) a first container having Aβ amyloid protein immobilized upon the inner surface, b) a second container which contains laminin-derived fragments or laminin-derived polypeptides dissolved in solution, c) a third container which contains antibodies specific for said laminin-derived fragments or laminin-derived polypeptides, said antibodies dissolved in solution, and d) a fourth container which contains labeled antibodies specific for laminin-derived fragments or laminin-derived polypeptides, said antibodies dissolved in solution.

With regard to systems and components above referred to, but not otherwise specified or described in detail herein, the workings and specifications of such systems and components and the manner in which they may be made or assembled or used, both cooperatively with each other and with the other elements of the invention described herein to effect the purposes herein disclosed, are all believed to be well within the knowledge of those skilled in the art. No concerted attempt to repeat here what is generally known to the artisan has therefore been made.

In compliance with the statute, the invention has been described in language more or less specific as to structural features. It is to be understood, however, that the invention is not limited to the specific features shown, since the means and construction shown comprise preferred forms of putting the invention into effect. The invention is, therefore, claimed in any of its forms or modifications within the legitimate and valid

scope of the appended claims, appropriately interpreted in accordance with the doctrine of equivalents.

Table 1

Summary of the effects of Peptides on Ab42 fibril

Analyzed at Ab42:Peptide wt/wt ratio of 1:2

				% Loss of 218 CD ellipticity
1.	RKRLQVQLSIRT #	AG73 or A1	12aa	-21.6 (gain)
2.	RQVFQVAYIIIKA ###	A13	13aa	37.5
3.	YLSKGRLVFALG	HA3G76	12aa	58.5 *
4.	TLFLAHGRLVFM ##	A4G82	12aa	85.6 ****
5.	AGQWHRVSVRWG	A5G81	12aa	91.6 ******
6.	DGRWHRVAVIMG	A5G101	12aa	89.0 *****
7.	HQTWTRNLQVTL	HA3G47	12aa	-20.2 (gain)
8.	ISNVFVQRLSLS	HA3G58	12aa	3.85
9.	RGLVFHTGTKNSF	HA3G74	13aa	34.4
10.	GNSTISIRAPVY	HA3G83	12aa	30.5
11.	VRWGMQQIQLVV	A5G82	12aa	27.6
12.	APVNVTASVQIQ	A5G109	12aa	14.9
13.	TRISLQVQLRKR #	rAG73 or A1	12aa	43.3
14.	AKIIIYAVQFVQR ###	rA13	13aa	7.0
15.	GLAFVLRGKSLY	rHA3G76	12aa	76.0 ***
16.	MFVLRGHALFLT ##	rA4G82	12aa	48.1
17.	GWRVSVRHWQGA	rA5G81	12aa	58.6 **
18.	GMIVAVRHWRGD	rA5G101	12aa	48.8